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PLANT AND SOIL

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PLANT AND SOIL

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ON THE EFFECT OF FERROUS SULPHATE ON THE AVAILABLE MANGANESE IN THE SOIL AND THE UPTAKE OF MANGANESE BY THE PLANT

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INTRODUCTION

Since 1947 a considerable number of field trials, testing the need for various trace elements have been made in the province of Lolland–Falster (H o l m e H a n s e n) [3] where the arable land consists mainly of heavy limed loams. In these trials iron and manganese have been tested separately at several levels as ferrous sulphate and manganese sulphate. The results of a number of such trials using sugar beet as the test crop are summarised in Table I.

TABLE I

Mean increases in yields of sugar beet from application of manganese or ferrous sulphate to soils

Year	Number of experiments	Kg per ha		Hkg of roots per ha		
				Mean yields non-treated plots.	Mean increase in yields	
		$MnSO_4 \cdot H_2O$	$FeSO_4 \cdot 7H_2O$		$MnSO_4 \cdot H_2O$	$FeSO_4 \cdot 7H_2O$
1947	4	50	50	362	17.0	12.8
1948	6	50	50	418	9.7	11.8
1949	16	25	50	379	7.6	2.9
1950	21	25	100	406	10.3	8.3
1951	17	25	100	373	—2.5	—1.8
1952	10	25	100	321	3.3	1.1

The figures for the increase in yield given in Table I are averages of greatly varying individual values. From these results it must be considered as established that application of ferrous sulphate

has in a number of cases given a considerable increase in the yield of sugar beet.

Since symptoms of iron deficiency were not observed in any of these trials and since iron deficiency in crops has been found only very rarely under Danish farming conditions it would seem doubtful whether the observed increases in yield of sugar beet after application of ferrous sulphate were caused by an increase in available iron.

It is further evident from the values in Table I that there is some agreement between the increase in yield for manganese sulphate added and that for ferrous sulphate added since increases in yield in general move in the same way from year to year.

Signs of manganese deficiency were common and widespread on the soils in these trials. The soil reaction in general was neutral or alkaline except for two soils which had a pH below 7.0. There thus existed a possibility that the effect observed on application of ferrous sulphate may have been an indirect one, the primary effect being due to manganese. Addition of ferrous sulphate could influence the manganese status of the soil in two ways; by oxidation of Fe^{++} ions to Fe^{+++} ions whereby at the same time higher oxides of manganese may be reduced, and also the ferrous ion might itself release bivalent manganese from soil colloids by exchange reaction.

METHODS

The *pot experiments* were carried out in cylindrical zinc pots, coated on the inside with paraffin wax. The pots were supplied with external pipes for watering from the bottom of the container. Broken flints were used for draining. The pots contained 20–22 kg mineral soil and had a surface area of 500 sq. cm.

The *manganese content of crops* was determined colorimetrically as permanganate after wet ashing with a mixture of sulphuric- and perchloric acid and oxidizing divalent manganese in the neutralized solution with potassium persulphate in the presence of sulphuric acid and silver ions.

The *content of iron in the crops* was determined according to the method of Szalkowski and Frediani[10]).

Nitrogen in the crops was determined by a micro-Kjeldahl method, using a modification of Parnas and Wagner's apparatus.

The *"exchangeable" manganese of soils* was estimated by shaking 50 g soil with 200 ml *M* magnesium nitrate for 1 hour. Manganese was then determined colorimetrically in the filtrates as permanganate after oxidation with potassium persulphate.

All *pH measurements* were made with the glass electrode.

The estimation of the contents of available phosphorus (Ft), potassium (T_K), manganese (T_{Mn}), and copper (T_{Cu}) in the soil was made according to methods used in Danish advisory work.

EXPERIMENTAL RESULTS

a. Pot experiments

Pot experiments in 1950 were designed to test the effect of application of iron as ferrous sulphate or chloride to a soil which was simultaneously fertilized with considerable amounts of manganese. The soil, a heavy loam from Kærstrup in Lolland, was taken from the non-treated plots of a field experiment where in 1949 the addition of iron and manganese was tested. The increase in yield following application of 50 kg of ferrous sulphate was, in that year, 29 hkg of root per ha. At the beginning of the experiment the soil had a pH value of 7.7, and the values of available nutrients were Ft 5.3, T_K 3.8, T_{Mn} 0.2, and the humus content was 3.0 per cent. While the soil was very low in available manganese it contained fairly good supplies of phosphorus and potassium. The soil was kept at a moisture content equal to 60% of its total water holding capacity calculated on an air-dry basis.

The experiment was carried out according to the design shown in Table II. A basal dressing of 15 g $NaNO_3$, 1.98 g KH_2PO_4, 2.52 g K_2HPO_4, 2.00 g $MnSO_4 . 4H_2O$, and 0.50 g $CuSO_4 . 5H_2O$ was added per pot.

TABLE II

Design of experiment 1950. Barley				
Treatment	g per pot			
$FeSO_4 . 7H_2O$	0	1.50	3.00	0
$FeCl_2 . 4H_2O$	0	0	0	2.14 *)
Number of replications	5	5	5	5

*) Same amount of Fe as in 3 g $FeSO_4 . 7H_2O$.

All materials were applied as solutions.

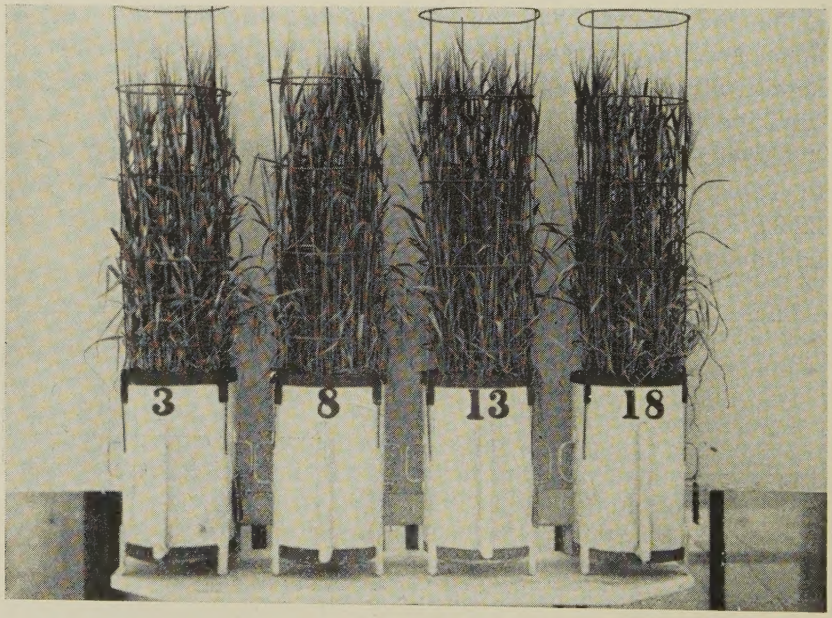
The test crop was barley *var.* Carlsberg.

Notes on plant growth

The pots were sown on 13/4, the barley germinated uniformly, and germination was completed by 27/4. On 15/5 the stock of plants was thinned to 25 plants per pot. During the summer the plants were repeatedly

sprayed with 1‰ nicotine solution to control aphids. In June a slight attack of mildew was observed but this did not apparently check growth. The barley tillered vigorously and grew well during the whole of the growth period. Ears started to form on 19/6 on the plants in the pots receiving 3.00 g ferrous sulphate, and on the following day on the plants in all the other pots. The earing was finished in all treatments on 27/6.

Until the beginning of June no differences in growth were observed in the various treatments. About the 10th of June the plants from non-treated pots showed slightly more erect growth and felt softer when handled than plants in the iron treatment pots. The plants fertilized with ferrous chloride were not quite so vigorous as those fertilized with ferrous sulphate. The most vigorous growth was obtained in pots receiving 3 g ferrous sulphate. These relatively small differences in growth disappeared in July, when growth was vigorous and uniform in all pots. Fig. 1 illustrates plant growth in pots representing all treatments on the 4th of July.



Without iron	1.5 g ferrous sulphate	3.0 g ferrous sulphate	2.14 g ferrous chloride

Fig. 1. Barley, 4/7. 1950

During the whole of the growth period symptoms of nutrient deficiency were not observed.

The crops were harvested on 5/8. The yields and the increase in yields are shown in Table III.

TABLE III

Yield of dry matter. Barley 1950				
Treatment	Yield, g dry matter per pot		Increase in yield, g dry matter per pot	
	Grain	Straw	Grain	Straw
Control	50.0	54.3	—	—
1.50 g $FeSO_4 . 7H_2O$	45.2	54.6	—4.8	0.3
3.00 g $FeSO_4 . 7H_2O$	49.8	52.2	—0.2	—2.1
2.14 g $FeCl_2$	50.8	51.8	0.8	—2.5
S.E. ±	2.4	1.2	3.4	1.7

It will be seen that there are no significant differences in the yields from various treatments or in the yields from the two levels of added iron under the experimental conditions used.

Total manganese and iron content of grain and straw are set out in Table IV.

TABLE IV

Uptake of manganese and iron. Barley 1950								
Treatment	Uptake per pot				Increased uptake per pot			
	Grain		Straw		Grain		Straw	
	mg Mn	mg Fe	mg Mn	mg Fe	mg Mn	mg Fe	mg Mn	mg Fe
Control	0.745	3.8	0.863	215.0	—	—	—	—
1.50 g $FeSO_4 . 7H_2O$	0.673	3.5	0.868	190.0	—0.072	—0.3	0.005	—25.3
3.00 g $FeSO_4 . 7H_2O$	0.842	3.5	0.950	183.2	0.097	—0.3	0.087	—32.1
2.14 g $FeCl_2 . 4H_2O$	0.869	3.7	0.979	184.9	0.124	—0.1	0.116	—30.2

The manganese content increased when iron was added to the soil. The increases were similar for grain and straw. No corresponding increase, however, was found in total iron content while a decrease in total iron content of straw was found for increasing amounts of iron applied to the soil. The iron content of the grain was hardly effected by increasing the application of iron to the soil.

In 1951 pot experiments were carried out as shown in Table V. The uptake of manganese, iron, and nitrogen by sugar beet was determined at maturity.

TABLE V

Design of experiment 1951. Sugar beet								
Treatment	1	m	f	n	mn	mf	fn	mfn
g $MnSO_4 . 4H_2O$ per pot	0	2	0	0	2	2	0	2
g $FeSO_4 . 7H_2O$ per pot	0	0	3	0	0	3	3	3
g $NaNO_3$. per pot	0	0	0	10	10	0	10	10
Number of replications	9	9	9	9	9	9	9	9

A basal dressing of 2.0 g KH_2PO_4, 2.5 g K_2HPO_4, 0.1 g $Na_2B_4O_7$, 2.0 g $NaNO_3$ (Faarevejle) and 3.6 g $NaNO_3$ (Købelev) was added to the soil in each pot. All nutrients were added as solutions. The following treatments in addition to the above were also included. Plants in 4 spare pots were repeatedly painted with a weak solution of ferrous sulphate. Similar applications of a solution of manganese sulphate of the same strength were given to the plants in 4 other spare pots.

Two soils were used. One from Faarevejle, Sjælland, was a highly organic soil similar in type to muck soils. It was taken from an area where crops show manganese deficiency. The other soil from Købelev, Lolland, was a heavy loam taken from the non-treated plots of a field trial in which in 1950 addition of iron and manganese was tested. In these (1950) field trials increases in roots and tops of 40 and 7 hkg respectively had been obtained from application of 25 kg manganese sulphate per ha, and increases of 50 hkg roots and 14 hkg tops from application of 100 kg ferrous sulphate per ha.

The results of soil analyses for available nutrients are summarised in Table VI.

TABLE VI

Soil reaction, available phosphorus, potassium, manganese, copper, and humus content of soils used in the 1951 experiment						
Soil	pH	Ft	T_K	T_{Mn}	T_{Cu}	% humus
Faarevejle	6.9	8.5	13.2	0.2	15.5	14.4
Købelev	8.1	7.5	8.8	0.2	17.2	2.9

The soils used were poor in exchangeable manganese, but had an adequate supply of available phosphorus, potassium, and copper.

On the 9th of May the pots were sown with sugar beet seed, which had kindly been placed at our disposal by A/S De danske sukkerfabrikker (The Danish Sugar Factories, Ltd.), the sample Type N having been taken

from the mixture delivered to the sugar beet growers from Maribo Sugar Factory in 1951. On account of genetic differences it is generally more satisfactory to use only seeds from one beet plant for pot experiments; but in the present case it was desirable to use the same mixture of seed as that used commercially in the year in question.

The plants were thinned gradually in such a way that by the end of June there was one plant left in each pot. In July the plants in 4 of the spare pots were painted 5 times with a 3‰ manganese sulphate solution and the plants in 4 other spare pots with a 3‰ ferrous sulphate solution. In August the plants were painted 3 times, the strength of the solutions being reduced to 1.5‰. During the summer the plants were sprayed repeatedly with a 1‰ nicotine solution to control aphids. The pots were watered to a moisture content equivalent to 60% of total water holding capacity calculated on an air-dry basis. During the latter part of the growing season the moisture content of the soils was increased and was held at about 80 and 90% for the Faarevejle and Købelev soils respectively.

Note on plant growth

Slight signs of manganese deficiency were observed in plants from all non-treated pots and in plants painted with Fe solution in the Købelev soil series. The plants in all the other treatments were healthy. Manganese deficiency symptoms were more pronounced in the pots containing Faarevejle soil and were scored at 2/8. The scoring was in arbitrary units, 0 being healthy plants and 1–3 increasing degree of intensity of manganese deficiency. The results are summarised in Table VII.

The plants which had been painted with manganese sulphate had a fine green colour, while those painted with ferrous sulphate showed

TABLE VII

Scoring of intensity of manganese deficiency of sugar beet leaves from various soil treatments Faarevejle 1951

Treatment	Mean scores *)
l	3.0
m	1.0
f	2.7
n	2.8
mn	0.3
mf	0.3
fn	0.8
mfn	0

*) Scoring in arbitrary units (0 = healthy plants, 1–3 degrees of intensity).

symptoms of the same type and intensity as the untreated sugar beets.

During late summer and autumn the intensity of the symptoms of manganese deficiency decreased in plants growing on both soils. At harvest time (October 1st) the beet plants growing on the Købelev soil were free from signs of manganese deficiency, whilst pronounced symptoms still remained in the beet plants growing on the Faarevejle soil. The effect of manganese on yield was thus smaller at harvest time than was expected from the observations made in July.

Applications of manganese sulphate and ferrous sulphate had no effect on the yield of sugar beet on the Købelev soil, and yield values are therefore given only for Faarevejle soil in Table VIII.

TABLE VIII

Mean yields of dry matter of sugar beet. Faarevejle 1951

Treatment	Yield of dry matter, g per pot		Increase in yield of dry matter, g per pot	
	Root	Top	Root	Top
0	66.9	31.5	—	—
m	79.1	32.6	12.2	1.1
f	60.6	29.5	—6.3	—2.0
n	120.3	54.2	53.4	22.7
mn	141.8	53.9	74.9	22.4
mf †)	65.3	32.9	—1.6	1.4
fn	132.7	61.6	65.8	30.1
mfn	138.6	57.0	71.7	25.5
S.E. ±	5.7	2.0	8.1	2.8

†) Severe attacks by wireworms (*Elateridae*).

Table IX shows the mean effects of manganese, iron and nitrogen with their four components derived from Table VIII.

TABLE IX

Crop responses to application of manganese, iron and nitrogen to sugar beet Faarevejle 1951

Effect of	g dry matter per pot		Effect of	g dry matter per pot		Effect of	g dry matter per pot	
Manganese	Root	Top	Iron	Root	Top	Nitrogen	Root	Top
m–l	12.2	1.1	f–l	—6.3	—2.0	n–l	53.4 *)	22.7 *)
mf–f	4.7	3.4	mf–m	–13.8	0.3	mn–m	62.7 *)	21.3 *)
mn–n	21.5 *)	—0.3	fn–n	12.4	7.4 *)	fn–f	72.1 *)	32.1 *)
mfn–fn	5.9	—4.6	mfn–mn	—3.2	3.1	mfn–mf	73.3 *)	24.1 *)
Mean	11.1	—0.1	Mean	—2.7	2.2	Mean	65.4 *)	25.1 *)

*) The 0.05 level.

In the case of the Faarevejle soil there was a highly significant effect on the roots resulting from the nitrogen added and also a mean effect from manganese derived largely from this component in the presence of nitrogen (21.5*). The only significant difference in the case of iron was a more pronounced effect in the presence of nitrogen than in the presence of manganese. In the case of the tops there was a highly significant mean effect following addition of nitrogen and following the addition of iron but only in the presence of additional nitrogen as well. The mean effects of iron on both roots and tops were not significant.

The crops were analyzed for total contents of manganese, iron, and nitrogen. The results are given in Table X.

TABLE X

Uptake of manganese, iron and nitrogen in dry matter. Sugar beet 1951

Treatment	Faarevejle						Købelev					
	mg Mn per pot		mg Fe per pot		g N per pot		mg Mn per pot		mg Fe per pot		g N per pot	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
l	0.78	0.70	13.4	28.4	0.47	0.65	1.11	1.09	11.4	37.7	0.51	0.52
m	1.53	1.21	10.6	30.6	0.51	0.66	1.65	1.34	8.0	33.9	0.51	0.51
f	0.65	0.76	8.3	26.9	0.42	0.67	1.42	1.21	8.3	30.6	0.51	0.51
n	1.42	1.34	15.0	52.6	1.03	1.14	1.82	1.94	20.9	54.6	1.19	1.23
mn	2.59	1.75	17.4	44.5	1.04	1.16	2.43	2.00	14.6	60.6	1.21	1.12
mf	1.39	1.29	8.9	29.2	0.42	0.63	1.22	1.07	7.9	27.2	0.42	0.49
fn	1.82	2.16	14.7	59.0	1.07	1.40	2.05	1.96	14.8	58.3	1.20	1.06
mfn	2.83	2.12	14.8	56.3	1.12	1.17	2.78	2.11	22.5	62.9	1.20	1.21

On the basis of the figures in Table X it is possible to calculate the increased uptake of manganese, iron, and nitrogen, respectively, when these nutrients were added separately or combined with the two others. The effects of the various treatments on the increase in uptake of manganese, iron and nitrogen by sugar beet are set out in Table XI.

From Table XI it will be seen that the mean increase in uptake of manganese rose when manganese, iron, and nitrogen were added to the soil. The greatest rise was produced by nitrogen, and the lowest by iron. Furthermore it is seen, when the soils were treated with various components of iron, that the increase in uptake of manganese was highest in the manganese deficient Faarevejle soil when iron was added together with nitrogen. The

TABLE XI

Effects of various treatments on the increases in uptake of manganese, iron and nitrogen by sugar beet. 1951

Effect of manganese	Faarevejle		Købelev		Effect of iron	Faarevejle		Købelev		Effect of nitrogen	Faarevejle		Købelev	
	Root	Top	Root	Top		Root	Top	Root	Top		Root	Top	Root	Top
Increases in uptake of manganese, mg Mn per pot														
m–l	0.75	0.51	0.54	0.25	f–l	—0.13	0.06	0.31	0.12	n–l	0.64	0.64	0.71	0.85
mf–f	0.54	0.53	—0.20	—0.14	mf–m	—0.34	0.08	—0.43	—0.27	mn—m	1.06	0.54	0.78	0.66
mn–n	1.17	0.41	0.61	0.06	fn–n	0.40	0.82	0.23	0.02	fn–f	1.17	1.40	0.63	0.75
mfn–fn	1.01	—0.04	0.73	0.15	mfn–mn	0.24	0.37	0.35	0.11	mfn–mf	1.64	0.83	1.56	1.04
Mean	0.87	0.35	0.42	0.08	Mean	0.04	0.33	0.12	—0.01	Mean	1.13	0.85	0.92	0.83
Increases in uptake of iron, mg Fe per pot														
Mean	0.00	—1.58	—0.60	0.73	Mean	—2.43	3.83	—0.35	—1.83	Mean	5.18	24.33	9.30	26.88
Increases in uptake of nitrogen, g N per pot														
Mean	0.03	—0.06	—0.02	0.00	Mean	—0.01	0.07	—0.02	—0.03	Mean	0.61	0.56	0.71	0.65

mean increases in uptake of iron and nitrogen only rose when the soil was dressed with nitrogen.

b. Soil analyses

The soils used in the pot experiments were extracted with magnesium nitrate in the presence or absence of 1 mg ferrous sulphate per g soil. The extractions were made over a pH range 2–8 by

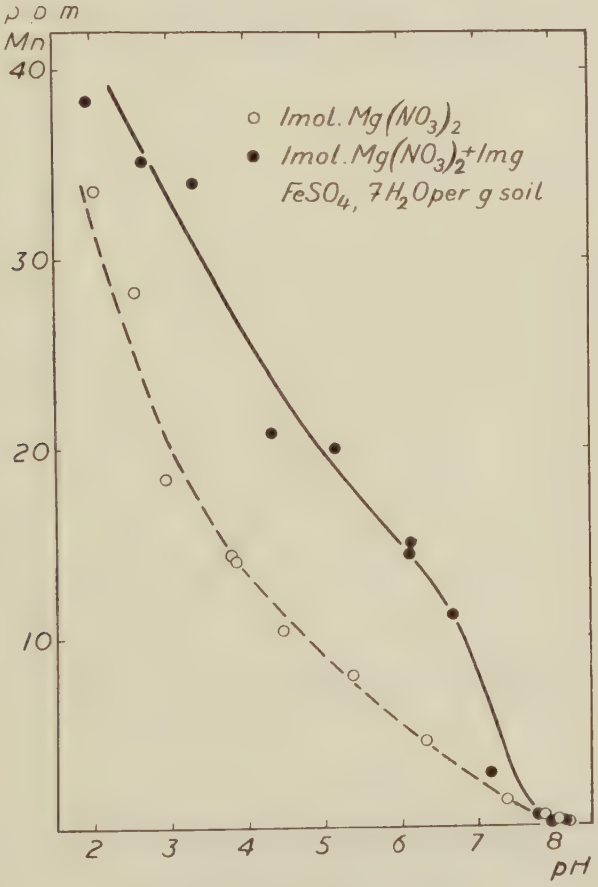


Fig. 2. Kærstrup.

addition of nitric acid or sodium hydroxide to the extracting agent. The results are presented in Figures 2, 3 and 4, where the lower curve shows the content of "exchangeable" manganese in

the soil without addition of iron, and the upper curve shows the content of "exchangeable" manganese in the soil when the magnesium nitrate solution used contains ferrous sulphate.

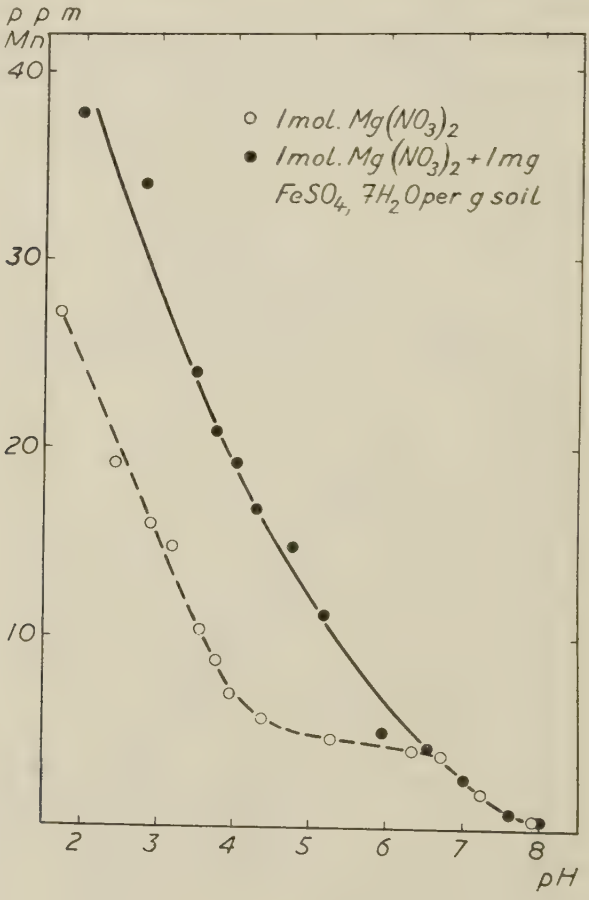


Fig. 3. Faarevejle.

From the results it is evident that the amount of "exchangeable" manganese can be considerably increased by addition of ferrous sulphate to the extracting agent.

DISCUSSION AND CONCLUSIONS

Results from a pot experiment in 1950 using a soil which, under field conditions, had given increased yields following application

of ferrous sulphate showed (Table III) that if sufficient amounts of available manganese were present further additions of ferrous sulphate had no effect on the yield. It seemed likely, therefore,

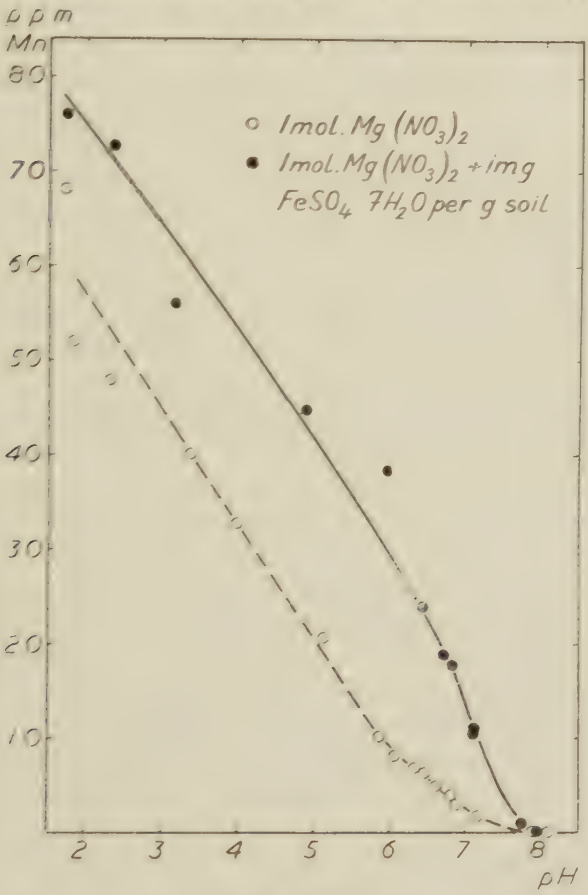


Fig. 4. Købelev.

that the effects observed following application of ferrous sulphate in the field trial might be due to an increase in available manganese.

An effect of manganese when ferrous sulphate is added may also explain the findings of H i l t n e r and K o r f f [4] in 1916 in pot experiments with oats using five soils deficient in manganese. Considerable increases in fresh weights and number of grains were found both when the soils were dressed with ferrous sulphate and when they were dressed with manganese sulphate.

If an application of ferrous compounds in certain cases may have the same effect as addition of manganese, this must of course mean that the plants absorb more manganese as a consequence of such treatment. In the experiments in 1950 it is seen (Table IV) that addition of ferrous sulphate or ferrous chloride to a soil not deficient in manganese causes the uptake of manganese to be increased even in the case of barley which grew vigorously.

The effect of nitrogen deficiency in the 1951 experiments overshadowed the manganese deficiency in both soils. Presumably the effects of applications of iron would have been clearer if higher nitrogen dressings had been used.

The symptoms of manganese deficiency in sugar beets on the Købelev soil, as mentioned above, disappeared completely during the late summer, and there was no increase in yields of dry matter at harvest time in those cases where manganese or ferrous sulphate had been applied. But if it may be assumed that the effect of ferrous sulphate is through a manganese effect, an effect of ferrous sulphate was also not to be expected. In the case of the Faarevejle soil (Table IX) there was a significant increase in yield of top and an effect on roots after addition of ferrous sulphate when the soil was at the same time fertilized with nitrogen. If both nitrogen and manganese were added to the soil the application of ferrous sulphate did not produce any increase in yield. The conclusion that the effect of ferrous sulphate is to be regarded as an effect on manganese availability is supported by the fact that the observed symptoms of manganese deficiency disappeared when the plants were painted with manganese sulphate. The plants were not deficient in iron since leaves repeatedly painted with ferrous sulphate did not change their appearance.

From the experiments made in 1951 (Table XI) it is seen that the effects on the manganese uptake by sugar beet following addition of ferrous sulphate to the manganese deficient Faarevejle soil were highest in the presence of nitrogen. In the case of the Købelev soil, which is not manganese deficient, increases in manganese uptake were positive for all combinations of treatments except when iron was added together with manganese.

It is of interest to note that application of ferrous sulphate only increased the uptake of manganese on the Købelev soil and was without effect on the manganese deficient Faarevejle soil. The

difference could be caused by the manganese being more firmly held in the Faarevejle soil which was rich in humus as compared with the Kobelev soil the latter having a humus content similar to that for average Danish soils.

A similar increase in uptake of manganese by the plants, following the addition of ferrous sulphate to mixtures of soil and sand, has been found by Schmehl *et al.* [7]) in pot experiments with lucerne and by McGeorge [5]) in Neubauer tests. In a pot experiment with soy beans grown on a soil deficient in manganese Mann [4]) also found an increase in the uptake of manganese by the plants when ferrous chloride was added to the soil.

It has been recorded by Sommers and Shive [9]), Sideris *et al.* [8]), Morris and Pierre [6]) that in water-culture experiments made with various plants and at different levels of yield, there is no increase in the uptake of manganese — or there may even be a decrease in the uptake of manganese — as a result of an addition of ferrous sulphate. It must therefore be assumed that the increased uptake of manganese by the plants as a result of addition of ferrous sulphate must be due to the effect of this fertilizer on the growth medium used here, viz. soil. Laboratory investigations (Figs. 2, 3 and 4) show that the content of exchangeable manganese of the experimental soils was considerably increased over a pH range 2–8 when ferrous sulphate was added to the extractant. This agrees with the finding of Heintze and Mann [1]) that "the uptake of added divalent manganese by soil in the presence of ammonium acetate at pH 7 was markedly reduced by ferrous ammonium sulphate, but not by the corresponding ferric salt".

From the results it is not possible to reach a decisive conclusion as to whether the increase in exchangeable manganese in the presence of ferrous sulphate is due to an exchange reaction or reduction reaction or both although investigations in progress indicate that the reduction reaction may be the most important.

SUMMARY

Application of iron as ferrous sulphate or chloride to a loam not deficient in manganese had no effect on the yield but increased the uptake of manganese even in barley which grew vigorously.

In an experiment with sugar beet on two soils contrasted with regard

to their available manganese supply applications of manganese iron and nitrogen were tested in all combinations. On the Kobelev soil, not deficient in manganese, no increases in yields were obtained on addition of ferrous sulphate while increases in manganese uptake were found for all combinations of treatments except where iron was added in the presence of manganese.

On the manganese deficient Faareveje soil significantly higher increases in yields of roots were obtained from ferrous sulphate in the presence of nitrogen than in the presence of manganese. The effect of iron in the presence of nitrogen on the yield of tops was also significant. These treatments also gave the highest increases in manganese uptake.

The amounts of manganese extractable from the soils by magnesium nitrate over a range of pH 2–5 could be increased considerably by addition of ferrous sulphate.

The results support the suggestion that application of ferrous sulphate to some soils has the same effect as an addition of manganese.

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LIST OF LITERATURE

1) Heintze, S. G. and Mann, P. J. G., A study of various fractions of the manganese of neutral and alkaline organic soils. J. Soil Sci. **2**, 234–242 (1951).

2) Hiltner, L. and Korff, G., Über die Wirkung verschiedener Bodenbehandlungsmittel, besonders des Mangansulfates, auf das Wachstum des Hafers. Prakt. Blätt. Pflanzenbau u. Pflanzenschutz, **15**, 49–56 (1917).

3) Holme Hansen, H. H., Planteavlen på Lolland-Falster 1947, 1948, 1949, 1950, 1951 and 1952.

4) Mann, H. B., Availability of manganese and of iron as affected by applications of calcium and magnesium carbonates to the soil. Soil Sci. **30**, 117–142 (1930).

5) Mehlbach, W. F., Nutrient requirements of grain sorghums as determined by sending tests and field experiments. Arizona Agr. Exp. Sta. Tech. Bull. No. 115, 297–338 (1948).

6) Morris, H. D. and Pierre, W. H., The effect of calcium phosphorus and iron on the tolerance of lespedeza to manganese toxicity in culture solutions. Soil Sci. Soc. Amer. Proc. **12**, 382–386 (1947).

7) Schmehl, W. R., Peech, Michael and Bradfield, Richard, Causes of poor growth of plants on acid soils and beneficial effects of liming. I. Evaluation of factors responsible for acidoid injury. Soil Sci. **70**, 345–361 (1950).

8) Stiehs, C. F., Volk, H. Y. and Krauss, F. H., Effects of iron on the growth and ash constituents of *Ananas Comosus* (L.) Merr. Plant Physiol. **18**, 608–632 (1943).

9) Somers, I. I. and Shive, J. W., The iron-manganese relation in plant metabolism. Plant Physiol. **17**, 582–602 (1942).

10) Stallybrass, Edith, N. and Pyddoke, H. A., Determination of iron in wheat flour and bread. Cereal Chem. **27**, 1–7 (1950).

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SAWDUST COMPOSTS IN SOIL IMPROVEMENT *)

III. POT CULTURES STUDIES WITH COMPOSTS FROM a) OUTDOOR PITS b) WOODEN BINS WITH HIGH MOISTURE c) OTHER MIXTURES

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This report on experiments with composts of wood wastes is a continuation of studies previously published [1, 2]. Results are presented on greenhouse pot cultures and on cultures in outdoor plots. These contained as basic ingredients various compost mixtures, particularly wood shavings from outdoor pits and sawdust from outdoor bins.

EXPERIMENTAL

1. Greenhouse pot cultures with birch shavings composted in outdoor pits

The chief purpose of this series of experiments was to compare different types of manure and minerals as to effectiveness in decomposition of shavings, as well as to obtain some data on hardwood waste in comparison to previously used softwood, usually coniferous in origin. Casual observations reported by Hawkins [3] have shown that shavings decay in about one year when in contact with the soil, and that softwood, despite the name, takes about twice as long to rot, probably because of its high resin content.

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**) This report is based upon portions of a thesis presented in partial fulfillment of the requirements for the degree of M.S. at the University of New Hampshire.

The nine pits used for composting the shavings mixtures were dug three feet into the ground and the walls were bricked. Each was four feet wide by six feet long. The mixtures placed in them in the summer of 1950 were as follows:

Pit 1. Pure white birch shavings
Pit 2. $^1/_4$ cow manure, $^3/_4$ shavings
Pit 3. $^1/_4$ hen manure, $^3/_4$ shavings
Pit 4. $^1/_3$ shavings, $^1/_3$ fresh silage, $^1/_3$ mixture of cow, hen, horse manure
Pit 5. $^1/_4$ horse manure, $^3/_4$ shavings
Pit 6. $^1/_2$ shavings, $^1/_2$ silage
Pit 7. 5% 5-10-10 fertilizer, 5% ground limestone (30% $CaCO_3$), 90% shavings
Pit 8. 5% limestone, 25% cow manure, 70% shavings
Pit 9. 5% limestone, 25% hen manure, 70% shavings

All proportions were measured on a volume basis.

Late in October, 1951, over a year later, the composts were carefully examined. It was found that good decomposition had taken place in parts of the contents of all the pits but that none of the mixtures was completely decomposed. Poor drainage and the fact that part of the contents of each pit was under water a fair share of the time may largely account for this. Only the decomposed portions were used in the culture experiments here reported.

In all, eleven different crops were grown in pot cultures with these composts. There were three series of three successive crops, and one with two crops. The proportions of soil to compost with each crop series were as follows:

Series No.	% Compost	% Soil	Container *)	Crop No. 1	Crop No. 2	Crop No. 3
1	25	75	1 gal. can	Radish	Swiss chard	Buckwheat
2	25	75	14 qt. pail	Tomatoes	Beans	
3	100	0	1 gal. can	Radish	Barley	Buckwheat
4	20	80	1 gal. can	Radish	Swiss chard	Buckwheat

*) 1 gallon = 3.785 litres, 1 qt. = 0.9463 litres.

For each crop a good loam soil was used for a control and 10 containers were used for each compost mixture or for the controls with soil only. In cultures of smaller plants several were grown per container. Thorough mixing of soil and composts preceded their placement in containers. Nutrient analysis of all composts before

mixing with soil showed them to be low in nitrogen, but that a moderate amount of other nutrients was present. The pH averaged about 6.0. At the start of a series of successive crops a uniform and ample application of complete fertilizer was made. Prior to each succeeding crop, nutrient tests were run for each mixture and those nutrients which were low were supplied in what was estimated to be ample amount.

TABLE I

Yields from one series of successive crops in mixtures of shavings composts with soil
(Ten one-gallon cans per treatment)

Radish 1st crop	Mean of yield g dry wt.	*t* value in terms of control	Swiss chard 2nd crop	Mean of yield g dry wt.	*t* value in terms of control	Buck-wheat 3rd crop	Mean of yield g dry wt.	*t* value in terms of control
Pit 6	3.7 **)	4.50	Pit 9	15.1 **)	5.41	Control	12.9	—
3	3.1 **)	3.64	3	13.1 **)	3.34	Pit 4	12.2	0.76
4	3.1 **)	4.91	7	10.1	1.68	8	12.2	0.86
8	2.8 **)	3.83	6	9.3	0.71	2	12.2	0.92
9	2.7 *)	2.55	Control	8.7	—	9	11.7	1.25
2	2.4 *)	2.25	5	4.8	3.29	5	11.0	2.52
Control	1.2	—	4	3.2	5.89	3	9.2	3.22
5	0.9	3.78	8	2.9	7.62	7	9.2	5.09
1	0.3	6.69	2	1.3	10.55	6	8.0	4.69
7	0.04	7.71	1	0.2	5.02	1	1.3	16.98
Standard error range .0079–.3425			Standard error range .3365–1.578			Standard error range .1402–.9301		

*) Significant (5% level).
**) Highly significant (1% level).

The tabular data for yields from these cultures is too bulky to be presented in full here. A typical set of yields expressed as means for all the plants grown in a given treatment are presented in Table I for Series No. 1. The statistically significant yields above the control (soil only) yields is indicated there. The sequence of the treatments listed in each column is in the order of size of yields.

It is evident from Table I that there is considerable variation from one crop to another in the significantly higher yields for different treatments. For example, the yield from pit 6 mixture was highly significantly greater than that of the control with radish, but was not so for the other two crops. On the other hand, the yield from pit 3 mixture was highly significantly better for both

radish and Swiss chard. None of the compost mixtures produced better yields than the controls for the third crop in this series, buckwheat. This variation in relative position of the yields for the different composts was encountered in all of the other series of crops grown.

TABLE II

Frequency of occurrence of crop yields at significant levels above controls (Based on eleven crops grown in shavings compost mixtures)

Pit No.	Compost mixtures	Number of times	
		Highly significant (1% level)	Significant (5% level)
6	1/2 silage, 1/2 shavings	3	0
8	1/4 cow manure, 3/4 shavings + lime	3	0
3	1/4 hen manure, 3/4 shavings	2	3
9	1/4 hen manure, 3/4 shavings + lime	2	2
4	1/3 silage, 1/3 manure mixture 1/3 shavings	2	1
2	1/4 cow manure, 3/4 shavings	1	1
1	pure birch shavings	1	0
5	1/4 horse manure, 3/4 shavings	1	0
7	1/20 5-10-10 fertilizer 1/20 ground limestone 9/10 shavings	0	0

It seemed, then, that the only fair way of making an appraisal of these mixtures for their crop producing power was to tabulate the frequencies of significantly higher yields than controls for each of them. This has been done in Table II. There it is evident that the mixtures from pit 6, 8, 3, and 9 are the best of the group and that there is little difference between the four of them. It is noteworthy that hen manure and green vegetation are both good agencies for composting these shavings, which is in agreement with their effects previously noted on sawdust [6]). Comparison of the positions of mixtures of pit 8 in Table II with that of pit 2, indicates that lime has some beneficial effect in improving the composting qualities of cow manure. It is also interesting to note that the forms of plant nutrients in commercial fertilizers, as exemplified in the contents of pit 7, are not nearly so effective in producing a good compost as are the more organic manures. This treatment gave poorest yields of all.

2. Outdoor cultures with compost mixtures in wooden boxes

The results of experiments with field crops grown in a greenhouse do not always remain the same when these crops are grown under natural weather conditions. It seemed desirable to study the growth of plants in the shavings composts just described, in comparison to other wood wastes, under outdoor conditions. The field plots with corn, previously reported [2], gave results indicative of the possibilities of wood wastes in soil improvement, but the low amount of rainfall was a critical factor in some seasons. To have more control of this factor and still have outdoor growing conditions, large wooden boxes as containers were built near the greenhouse in June, 1952. Water could be added easily to these by hose when necessary. Each box was 6 feet long, 2 feet wide and 1½ feet deep. A partition was placed in the center of each, making two compartments 3 feet long. Holes in the bottom were provided for drainage and the boxes were placed on a bed of gravel to further aid in this respect. All future mention of the word "box" in this paper refers to the individual compartments.

Early in June, 1952, the boxes were filled with various mixtures of soil and organic materials. All of the composts, when used with soil, were thoroughly mixed with the soil. When sewage sludge and sawdust or hen manure and sawdust were mixed together, water was added to the manure or sewage and mixed until a pasty consistency was obtained, and then this pasty material mixed with the sawdust to insure an intimate mixture. For more details concerning the source of the sewage sludge, see next section.

Each of the boxes was filled to exactly 6 inches from the top, leveled and tamped evenly; 175 grams of fertilizer (5-10-10) was then broadcast over the mixture. The boxes were then filled to exactly 1½ inches from the top.

The mixtures used in these boxes were as follows:

1. 25% rotted sawdust, 75% soil
2. *Krilium* + soil (recommended mixture)
3. 2.5% sewage, 22.5% fresh sawdust, 75% soil
4. 10% sewage, 90% fresh sawdust
5. 2.5% sewage, 97.5% soil
6. 2.5% hen manure, 22.5% fresh sawdust, 75% soil
7. 2.5% hen manure, 97.5% soil
8. 2.25% sewage, 18.75% fresh sawdust, 75% soil
9. 25% sewage, 75% fresh sawdust

10. control (soil only)
11. 25% compost Pit 1, 75% soil
12. 25% compost Pit 2, 75% soil
13. 25% compost Pit 3, 75% soil
14. 25% compost Pit 4, 75% soil
15. 25% compost Pit 5, 75% soil
16. 25% compost Pit 6, 75% soil
17. 25% compost Pit 7, 75% soil
18. 25% compost Pit 8, 75% soil
19. 25% compost Pit 9, 75% soil
20. control (soil only).

Each of the above mixtures was duplicated once to fill the forty boxes, giving four replications for the controls.

Recently many soil conditioners have appeared on the market, among them K r i l i u m (Monsanto Chemical Co.). The object of using Krilium in these tests was to compare such a chemical soil conditioner with wood waste and composts as soil conditioners. The calcium salt form of Krilium was used, at a rate of 0.1% Krilium to 99.9% soil, based on a recommendation by P a r k s [7]). The Krilium was first mixed with about $\frac{1}{2}$ gallon of air-dried soil,

TABLE III

Relative crop response from compost mixtures in outdoor boxes.

Mixtures	Yield position (on basis of 4 crops)																			Accumulated position totals *)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
16	1	1	1			1														12
13	1	1			1			1												16
19		1	1		1					1										20
6	1	1					1				1									21
2			1			1		1		1										27
7	1			1								1	1							30
1					1		2					1								31
5					1			2			1									32
10					1				3											32 control
18				1						1	1			1						39
17			1						1				1			1				41
3				1						1		1					1			42
12						1							1	1		1				49
14						1						1		1				1		50
15											1	1			1	1				54
8													1	1	1	1				58
9							1										1	1	1	61
11															1		1		2	70
4																	1	2	1	72

*) (Example: for Mixture 16, 1 + 2 + 3 + 6 = 12).

and this in turn mixed with the entire volume of soil necessary to fill the two boxes.

The pit composts were the same ones described in the previous section.

For the first season three different crops were grown simultaneously, one row of each per box. They were rutabaga, rape and peas. In the second season onions were grown.

Again as with the pot cultures described in Section 1, the yields showed considerable variation in response to the different treatments from one crop to another. To save space the actual yield figures are not presented, but relative positions of the various mixtures based on yield response are shown in Table III. Based on this they are listed in that same order in Table IV, for ease in correlating components of mixtures with the numbers. Only in the onion harvest were yields taken in such a manner as to analyze them for significance. It seems evident, however, from Table III, that the combined yields for treatments 16, 13, 19 and 6 are probably significantly greater than the controls. For the onion crop 16 and 19 were highly significantly so (1% level) and 13 and 6 were significantly (5% level). Of these the relatively high yields for mixtures 16, 13 and 19 containing composts from pits 6, 3 and 9 respectively, were in close agreement with the response shown in Table II for those in pot cultures. It seems evident from this that both green vegetation (as typified in this instance by chopped fresh grass silage) and hen manure are remarkably good aids to composting of wood wastes. Krilium seems to be of questionable value based on this data. Sewage sludge and other manures than hen manure did not show any advantageous qualities as composting aids. The position of the compost produced by inorganic fertilizer (mixture 17, position 11) was again rather low.

Further crop growth tests with the materials in these boxes are being conducted.

3. Greenhouse pot cultures with sawdust, sewage and soil mixtures

Raw sewage and sewage sludge have been used as fertilizers for many years, particularly in Europe and in the Orient. In recent years much work has been done in the United States with sewage as a soil amendment, especially in the west. Fraps[3]) found that digested sewage sludge contains on the average about two-

TABLE IV

Sequence of excellence of crop response from compost and other mixtures in outdoor growing boxes. (Based on Table III)

Relative Rank	Mixture No.	Composition		
1	16	25% Pit 6:	50% silage 50% shavings	75% soil
2	13	25% Pit 3:	25% hen manure 75% shavings	75% soil
3	19	25% Pit 9:	25% hen manure 70% shavings 5% ground limestone	75% soil
4	6	2.5% hen manure 22.5% fresh sawdust		75% soil
5	2	0,1% Krilium		99.9% soil
6	7	2.5% hen manure		97.5% soil
7	1	25% rotted sawdust		75% soil
8	5	2.5% sewage		97.5% soil
9	10	Control		100% soil
10	18	25% Pit 8:	25% cow manure 70% shavings 5% ground limestone	75% soil
11	17	25% Pit 7:	5% fertilizer 5-10-10 90% shavings 5% ground limestone	75% soil
12	3	2.5% sewage 22.5% sawdust		75% soil
13	12	25% Pit: 2	25% cow manure 75% shavings	75% soil
14	14	25% Pit 4:	1/3 silage 1/3 shavings 1/3 horse, hen and cow manure	75% soil
15	15	25% Pit 5:	25% horse manure 75% shavings	75% soil
16	8	6.25% sewage 18.75% sawdust		75% soil
17	9	25% sewage 75% sawdust		
18	11	25% Pit 1:	100% birch shavings	75% soil
19	4	10% sewage 90% sawdust		

fifths as much available nitrogen as barnyard manure, and that activated sewage contains 10% more available nitrogen than barnyard manure. Since digested sewage sludge is easily obtained from the sewage plant in Durham, it seemed desirable to conduct some plant cultures using this sludge as an organic supplement to sawdust.

For the plant cultures the following mixtures were prepared, on a volume basis:

Mixture 1. 90% fresh sawdust
10% sewage sludge
Mixture 2. 1/3 (90% sawdust, 10% sewage sludge)
2/3 soil
Mixture 3. Soil only.

For Mixture 2, sawdust and sewage were first mixed in the same proportion as for Mixture 1 and then combined with soil at one part of the mixture to two parts of soil. Each set of cultures was supplied with an equal and adequate amount of complete fertilizer. The sewage as obtained from large drying beds was one year old. The sawdust was mostly softwood (coniferous). A summary of results on seven different crops is presented in Table V. Two successive crops were grown in two groups and three for the last one. The Swiss chard and buckwheat were grown in 1 gallon size cans and the others in 14 quart pails. Ten containers were used for each mixture.

TABLE V

Yields of crops grown in sawdust, sewage and soil mixtures (mean of yields — gram)

Crop	No. of successive crops	Mixture No. 1: 90% fresh sawdust 10% sewage sludge	Mixture No. 2: 1/3 (90% fresh sawdust 10% sewage sludge) 2/3 soil	Mixture No. 3: Soil only (control)
Corn	1	26.2 **)	23.3 **)	16.3
Tomato	2	161.6 (fresh wts of fruits)	186.2	216.6
Swiss chard	1	.44	10.8	9.6
Buckwheat	2	10.7 **)	5.6 **)	3.3
Sunflowers	1	9.0 **)	13.1 **)	6.6
Corn	2	33.5	49.7 *)	41.4
Corn	3	5.4	5.0	3.5

*) Significant (5% level.
**) Highly significant (1% level).

The results show highly significantly better yields than controls for three crops, and significantly better for one other with Mixture 2. For Mixture 1 three crops gave highly significantly better yields. The controls gave higher yields than both the other mixtures with

tomatoes, and better than Mixture 1 with two crops. In no case were the control yields significantly greater than other yields. It appears then that Mixture 2 was the better one of the sewage combinations.

4. Greenhouse pot cultures with mustard pulp and sawdust composts

In previously published work [6]) and in section 1 of this report there was some indication that green vegetation is a good composting aid for wood wastes. This has been suggested also by Bruce [1]) who has placed considerable emphasis on the use of juices or infusions from green vegetation as aids to composting. In order to test this point further, using the pulp of green mustard leaves, several composts were set up in the greenhouse. Leaves from vigorous mustard plants were ground to a pulp with a Waring blendor. This pulp and the resulting juices were mixed with various amounts of lime, fertilizer, manure and sawdust. The mixtures were placed in glass battery jars of five gallon capacity, two jars per treatment, as follows:

1. Sawdust only
2. 100 g limestone, 100 g 5-10-10 fertilizer, 100 g mustard pulp, 12 kilograms fresh sawdust
3. 100 g 5-10-10 fertilizer, 1000 g mustard pulp, 12 kilograms sawdust
4. 1000 g mustard pulp, 12 kilograms sawdust
5. 1800 g hen manure, 1000 g mustard pulp, 12 kilograms sawdust.

These mixtures were kept moist and stirred from time to time, for over a year. At that time, there appeared to be very little decomposition of the sawdust. Analysis for nutrients showed pH high, nitrogen quite low, but other nutrients fairly high.

Two months later, in January, 1952, these composts were mixed each with soil at the rate of 20% compost to 80% soil. The mixtures were then placed in 1 gallon size cans, ten cans per treatment and to each was supplied adequate fertilizer. Ten cans of soil only were used as controls and these were similarly supplied with fertilizer.

Cabbage plants were grown first in these mixtures, followed by corn. Preceding the corn, nutrient deficiencies in the mixtures were corrected as revealed by analysis following the cabbage. The yields are given in Table VI. All of the composts except sawdust only were highly significantly beneficial for the first crop, but for the second crop only mixture 5 showed benefit, possibly because of long lasting

nutrients in the hen manure. Probably the mustard pulp was rather completely decayed and had disappeared by the time of the second crop planting.

TABLE VI

Dry weights (grams) of crops from mustard pulpsawdust composts				
Mixture No.	1st crop, Cabbage		2nd crop, Corn	
	Mean of yield	*t* value compared to control	Mean of yield	*t* value compared to control
1	8.8	1.36	14.7	0.19
2	12.5 **)	8.38	14.3	0.47
3	11.6 **)	5.45	16.4	0.88
4	8.9 **)	3.53	12.4	1.82
5	13.6 **)	6.91	22.7 **)	4.25
6 control	4.2		15.0	

**) Highly significant (1% level).

5. *Greenhouse pot cultures with lime, fertilizer, and sawdust composts*

The results presented in section 1 of this report showed that commercial forms of fertilizers are not nearly so effective in composting of wood wastes compared to manures and green vegetation as shown by growth of crops in the resulting composts. In order to substantiate this more completely the following mixtures were prepared:

1. 2.5% fertilizer (5-10-10)
   2.5% slaked lime (30% $CaCO_3$)
   95% fresh sawdust
2. 1% fertilizer (5-10-10)
   1% slaked lime
   98% fresh sawdust
3. 5% slaked lime
   95% fresh sawdust
4. 100% fresh sawdust
5. 5% fertilizer (5-10-10)
   5% slaked lime
   90% fresh sawdust.

These mixtures were placed in large wooden tubs near the greenhouse, where they were stirred from time to time and kept moist for over 16 months. At this time there was very little visible evidence of decomposition. Nutrient analysis showed nitrogen to be quite low except for Mixture 5, which had a moderate amount

of NH_4 nitrogen. All of the other nutrients as well as pH, were high.

Each material was then mixed with soil at the rate of 20% compost to 80% soil, and placed in one gallon size cans, 10 per mixture. Soil also was prepared as a control. Each material was adequately fertilized at the start of each growth period.

TABLE VII

Dry weight yields (grams) of crops from lime, fertilizer and sawdust composts

Mixture No.	1st crop Cabbage		2nd crop Radish (fr. wt.)		3rd crop Buckwheat	
	Mean of yield	*t* value compared to control	Mean of yield	*t* value compared to control	Mean of yield	*t* value compared to control
1	2.02 **)	11.92	59.9	0.11	21.4	1.20
2	4.78 **)	6.67	61.1	0.17	24.8 †)	4.01
3	4.58 **)	6.32	64.4	0.22	17.8	1.61
4	4.18 **)	6.92	63.9	0.13	13.4 **)	6.44
5	1.97 **)	9.62	64.9	0.18	10.3 **)	8.28
6 (control)	8.77		49.2		20.0	

**) Highly significantly less than control (1% level).

†) Highly significantly greater than control.

Three different successive crops were grown in these mixtures, the results of which are given in Table VII. The yields of the first crop for the composts were all highly significantly less than that for the controls. Those for the second crop were greater, but not significantly so. Only one material, Mixture 2, showed significantly greater yields than the controls for the third crop and the remainder were considerably less than the controls or close to that value. This set of experiments agrees conclusively with previous results, that commercial or more completely inorganic fertilizers are not effective composting agents. Whether the better effects of organic materials is due to forms of nitrogen or other nutrients present or to the forms of microorganisms present, which in turn provide an inoculum for decay, is a debatable question. It thas been stated by Hopkins [5]) that the answer to this question can only be determined by careful long-term investigations.

6. *Greenhouse pot cultures with sawdust composts from outdoor bins*

The purpose of these composts was to test a method of storing

and treating composting materials in more compact form which would be easier to handle than those hitherto used. The piles of composts described previously [6] [8] has disadvantages from both these standpoints, while the pits described in section 1 of this paper suffered from inadequate drainage. It was also difficult to handle and remove the material from these pits.

Six wooden compost bins were built near the greenhouse in the summer of 1951. Each was of cubical form, three feet each way, open at the top. They were made of rough lumber, and each had one side removable, so that the composts could be turned over easily at intervals. They could be kept thoroughly moist by hose from the greenhouse when necessary. The mixtures placed in them were as follows:

Bin 1. 75% sawdust, 25% hen manure
Bin 2. 85% sawdust,15% hen manure.
Bin 3. 85% sawdust, 15% hen manure, 20 pounds superphosphate
Bin 4. 50% sawdust, 25% hen manure, 25% chopped green clover and grass.
Bin 5. 50% sawdust, 25% hen manure, 25% chopped green clover and grass, 20 pounds superphosphate
Bin 6. 90% sawdust, 10% sewage sludge.

About a year later the contents of each bin were thoroughly remixed. At this time very little decomposition of the sawdust could be observed. However, the green materials in Bins 4 and 5 had completely decomposed.

Six months later, or eighteen months from the start of these composts samples were taken for nutrient analysis. All were low in nitrogen and potassium and high in pH. The composts were mixed with soil in 20 to 80 proportions as previously described, with soil as controls. Six crops, in two groups of three successive ones, were grown in them. Results appear in Table VIII. The first set of three crops were grown in pails and the second set in cans.

For ease in determining which of these mixtures is best and which is next best, etc., the number of times their highly significantly better yields occur in relative positions are given in Table IX. There it is evident that Bin 4 mixture containing sawdust, green material and hen manure is the best. Bin 5, which is next to it, differs from it in containing superphosphate, so it is evident that the latter conferred no particular advantage. Poorest of all the composts was

TABLE VIII

Dry weight yields (grams) of crops from bin composts plus soil						
Bin No.	1st crop, Corn		2nd crop, Tomatoes (fr. frts.)		3rd crop, Sunflowers	
	Mean of yield	*t* value compared to control	Mean of yield	*t* value compared to control	Mean of yield	*t* value compared to control
1	28.5 **)	10.04	622.2 **)	7.84	8.5 *)	2.50
2	26.9 **)	9.56	625.4 **)	7.95	1.8	0.93
3	29.8 **)	8.84	808.2 **)	10.45	18.2 **)	8.89
4	30.3 **)	9.30	813.3 **)	10.37	21.6 **)	10.50
5	32.0 **)	14.18.	711.8 **)	7.11	37.8 **)	27.48
6	12.0	4.79	118.0	0.60	2.6	1.78
control	6.6		95.7		1.5	
	1st crop, Radishes		2nd crop, Beans		3rd crop, Buckwheat	
1	51.0 **)	15.40	37.6 **)	11.37	7.1 **)	6.46
2	41.7 **)	8.39	41.9 **)	12.79	5.1 **)	5.67
3	45.5 **)	9.67	35.6 **)	9.19	4.6 **)	3.76
4	48.2 **)	12.65	43.1 **)	5.16	5.7 **)	5.06
5	42.5 **)	9.06	40.8 **)	5.82	4.5 **)	3.34
6	14.6 †)	5.04	26.8 *)	2.60	5.1 **)	4.49
control	22.3		15.9		2,4	

**) Highly significantly greater than control (1% level).
*) Significantly greater than control.
†) Highly significantly less than control.

TABLE IX

Relative rank of treatments highly significantly greater than controls in bin composts plus soil, 20% compost plus 80% soil (Based on Table VIII)						
Treatment position	1st	2nd	3rd	4th	5th	6th
Bin 4	2	4				
Bin 5	2		2	1		1
Bin 1	2			2	1	
Bin 3		1	3	2		
Bin 2				2	2	1
Bin 6			1			2

the sewage-sawdust mixture, which agrees with previous results on use of sewage. It is definitely of doubtful value.

Continuing tests of these mixtures are being made.

SUMMARY

1. Extensive tests of growth of plants in pot cultures were made with mixtures of hardwood shavings composts with soil. The shavings had been composted in outdoor pits dug in the soil. As aids to composting, various manures, green vegetation and commercial fertilizers, were used. Several

conclusions can be drawn from these experiments: a) Pits of this type are not good containers for composts unless adequate drainage is provided. Even then, poor handling conditions for the compost may make their use inadvisable. b) Hardwood shavings did not decompose faster than softwood sawdust. c) The superiority of both hen manure and green vegetation as aids to composting was again substantiated. d) Commercial or inorganic fertilizer was poor in this latter respect.

2. Further trials of these pit composts in comparison to other materials, such as Krilium, sewage sludge and sawdust, in mixtures with soil, were made in boxes for growing plants outdoors near the greenhouse. Further verification of the superiority of hen manure and green plant material as composting amendments was shown by crop yields in these materials. No particular advantage was shown for the use of Krillium. Sewage mixtures, as well as of composts with inorganic nutrients, gave poorer yields than controls.

3. Greenhouse pot cultures were conducted with mixtures of sewage sludge, sawdust and soil in comparison to soil only. With some crops, the sewage mixtures showed significantly better yields than the soil controls, but with others not. Therefore, the results are somewhat inconclusive as to the value of sewage sludge as an addition to fresh sawdust and soil or to soil directly.

4. The value of fresh, green, macerated plant tissue, as exemplified by that of mustard leaves, was tested as a composting aid. Its effects on sawdust, alone and in combination with lime and commercial fertilizer, was measured by growth of two crops in the resulting composts. For the first crop there was some benefit evident from the mustard pulp, but for the second these effects had disappeared. Only residual effects of the hen manure were significant. Probably the beneficial effects of green vegetation may be realized over a longer period of time if it is used in larger amounts and in less finely divided form.

5. A more complete test of the adequacy of commercial fertilizer as a composting agency was sought. This was done by means of mixtures of sawdust with various amounts of lime and complete fertilizer and measure of crop growth in the resulting composts. The results showed almost a total lack of value of these materials as composting aids. This difference in response compared to more organic forms might be due to differences in the forms or lasting qualities of nutrients present or to differences in microflora, or to both.

6. Some of the best of the previously tested sawdust compost mixtures were prepared and placed in containers of a different kind. These were outdoor, wooden bins near a source of moisture. One mixture of sawdust and sewage was prepared also. This kind of container was found to be much superior to either pits in the ground or piles on the ground previously used. Again crops in these composts showed best response when grown in mixtures of those prepared with green vegetation, hen manure, or both. That in sewage-sawdust was poor.

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REFERENCES

1) Bruce, Maye E., From vegetable waste to fertile soil; Quick return compost. Faber and Faber, Ltd., London (1943).
2) Dunn, S., Wolfe, L. P. Jr., Macdonald, W. A. and Baker, J. R., Field plot studies with sawdust for soil improvement. Plant and Soil **4**, 164–170 (1952).
3) Fraps, G. S., The composition and fertilizing value of sewage sludge. Texas Agr. Exp. Sta. Bull. 445 (1932).
4) Hawkins, A. D., Wood and soil husbandry. New England Homestead, July 10, (1948).
5) Hopkins, D. P., Chemicals, humus, and the soil. Faber and Faber, Ltd., London (1946).
6) Macdonald, W. A., and Dunn, S., Sawdust composts in soil improvement. II. Pot culture studies with compost mixtures of sawdust and manure, steam treated composts, and miscellaneous mixtures. Plant and Soil **4**, 235–247 (1953).
7) Parks, R. Q., Krilium. Science **115**, 3 (1952).
8) Wolfe, L. P. Jr., and Dunn, S., Sawdust composts in soil improvement I. Studies on aeration, acid hydrolysis, manure, and waste materials as composting aids. Plant and Soil **4**, 223–234 (1953).

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THE INTERRELATIONSHIPS OF IRON AND POTASSIUM IN THE POTATO PLANT *)

by E. W. BOLLE-JONES **)

INTRODUCTION

Evidence that iron and potassium are interrelated in the metabolism of the potato plant has been provided by preliminary experiments described by Hewitt and Bolle-Jones [9]) who suggested that potassium may enhance iron utilisation and that iron may impede the translocation of potassium. It was planned in the following pot-sand-culture experiments to make a more detailed study of the significance of these interrelationships in the potato and to find how these relationships were affected by the presence of calcium carbonate and varying levels of phosphate supply.

In the first of these experiments, carried out in 1948, potato plants were grown at varying levels of iron and potassium supply in the presence or absence of calcium carbonate. It was found that increased potassium supply increased the concentration of iron in the tubers and laminae but depressed the phosphorus and increased the chlorophyll concentrations of the laminae. The presence of calcium carbonate did not affect the nature of the relationships which existed between iron and potassium.

The results suggested, as the simplest working hypothesis, that the ameliorative effect of potassium on iron deficiency chlorosis might be explained in terms of its depressive effect on phosphorus concentration which increased the "availability" of the iron present in the lamina. Accordingly a factorial experiment designed to include varying levels of iron, potassium and phosphate was carried out to investigate this hypothesis in 1949. It was concluded as a result of

*) These studies were carried out at Long Ashton Research Station and comprise part of the data submitted in a Ph. D. thesis to the University of Bristol in 1952.

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this experiment that the ameliorative influence of increased potassium supply on iron deficiency chlorosis was partially due to the increased iron concentration of the laminae and partially to the increased efficiency of iron utilisation in chlorophyll formation. This increased efficiency was attributed not only to the decreased phosphorus concentration, *per se*, but also to the effect of potassium in reducing the ability of the phosphorus to immobilise the iron in the laminae.

Although increased potassium supply increased the concentration of iron in the roots, tubers and laminae it was not known whether a greater "absolute" amount of iron was taken into the plant. The effects observed may have been due to a redistribution of iron within the plant. A study of the relative concentrations of potassium in young and old laminae had also indicated that increased iron supply affected the distribution of potassium within the potato plant but it was not known whether it affected total potassium uptake. An experiment, carried out in 1950, was designed to obtain information on these points. In addition, preliminary investigations designed to test the theory that the role of potassium in mitigating the severity of iron deficiency was connected with its effect on the conversion of inorganic to organic phosphate and, possibly, to its influence on the formation of organic acids, were carried out in 1951.

The following account is a description of these experiments. Most of the incidental observations in the literature relevant to this study, have been cited elsewhere [9]).

EXPERIMENTAL

1. *Design of experiments*

In the 1948 experiment there were thirty-two treatments representing combinations of four levels of iron, four levels of potassium and two levels (with and without) of calcium carbonate. The iron and potassium levels were chosen to represent severe deficiency (Fe_1 and K_1), mild deficiency (Fe_2 and K_2), normal (Fe_3 and K_3) and luxury (Fe_4 and K_4) consumption. These concentrations are given in Table I. Each treatment received a supply of soluble calcium. In addition, one half of the pots (C_2 treatments) each received 50 g of "Analar" grade calcium carbonate which was well mixed with the sand (9½ kilograms per pot) prior to planting; treatments not receiving calcium carbonate were designated C_1. A certified Scotch seed tuber, variety Majestic, was planted in each pot on the 10th May 1948; each

treatment was carried out in duplicate and each replicate consisted of four pots.

The 1949 experiment was designed to include three levels each of iron, potassium and phosphate which were chosen to represent deficiency (Fe_1, K_1 and P_1), normal (Fe_2, K_2 and P_2) and luxury (Fe_3, K_3 and P_3) consumption. The concentrations of these nutrients are given in Table I. Each of the 27 treatments was carried out in duplicate and each replicate contained eight pots in each of which a tuber (variety Majestic) was planted on the 25th of May, 1949.

TABLE I

Nutrient levels of iron (Fe), potassium (K), and phosphate (P), expressed as milligram-equivalents per litre

	1948	1949	1950–51		1948	1949	1950–51		1948	1949	1950–51
Fe_1	0.0005	0.0024	approx. 0.0016	K_1	0.50	0.60	0.60	P_1	—	0.90	0.90
Fe_2	0.0075	0.0600	—	K_2	1.25	2.40	—	P_2	4.00	2.70	—
Fe_3	0.1125	1.50	1.00	K_3	3.12	9.60	9.60	P_3	—	8.10	8.10
Fe_4	1.6875	—	—	K_4	7.81	—	—		—	—	—

In the 1950 experiment iron, potassium and phosphate were each applied at two levels chosen to represent deficiency (Fe_1, K_1 and P_1) and luxury consumption (Fe_3, K_3 and P_3). The potassium and phosphate levels were identical with those of the 1949 experiment (Table I). There were eight replicates, one in each block; each replicate or plot consisted of four pots. A tuber, variety Majestic, was carefully washed, dried and weighed before planting in each pot on the 2nd of May 1950.

In 1951 a few selected treatments, chosen so as to reproduce some of the main visual effects observed in the previous experiments of this series, were applied with or without the addition of organic acids to the nutrients. The acids chosen (malic, citric and oxalic) were applied at a concentration of 10 me/l.

Throughout these experiments calcium, magnesium and nitrate concentrations were maintained constant at 8, 3 and 12–14 me/l respectively; the supply of known micronutrients to each treatment was also constant.

2. *Culture technique*

Bitumen painted pots, acid washed sand, demineralised water and nutrients as described by Hewitt [7]) were used. There was no evidence to show that increased potassium level carried iron, as an impurity, into the nutrient. This point is one of considerable importance in the assessment of the experimental results. The pH of the nutrient solution increased with iron level; hence at deficiency levels of iron the nutrient pH hindered rather than favoured the production of chlorosis.

3. *Sampling and analytical procedures*

i. Sampling. "Young" and "old" (Hewitt and Bolle-Jones[9]) leaflets were sampled throughout each season at monthly intervals. The midrib was excised from each lamina and discarded; the following weights of lamina were then rapidly weighed out: 0.5 g (dry weight); 0.5 g (chlorophyll); 1.0–3.0 g ("soluble" K, P, and Ca) and 0.5–2.0 g ("soluble" Fe). Samples for dry weight were oven-dried at 85°C for 36 hours and reweighed; samples for chlorophyll estimation were stored in solid carbon dioxide. Samples for "soluble", nutrient estimations were stored at 5°C. The laminae which remained after this apportionment were dried and used for the determination of total nutrient elements. New tubers were analysed for the 1948–1950 experiments as well as the roots of the 1949 and 1950 plants.

In the 1950 experiment all plants grown in two of the Blocks (7 and 8) were completely harvested on the 11th of June; another two Blocks (3 and 4) were similarly harvested on the 13th of July. Plants grown in Blocks 3, 4, 7 and 8 were not sampled during the growing period; any fallen leaves were carefully collected and added to the "bottom" region of the shoot at the time of harvest. The aerial shoots of each plant were, on harvesting, subdivided into three regions according to length:

(*a*) the *Top* region which constituted $^1/_5$,
(*b*) the *Middle* region — $^2/_5$,
(*c*) the *Bottom* region — which contributed the remaining $^2/_5$.

Each region was further separated into laminae and stems plus petioles prior to being oven-dried. The "young" laminae of the 1948–1951 monthly samplings, were taken from the top region whereas the "old" laminae were taken from areas which corresponded partly with the middle and partly with the bottom regions.

ii. Maceration. All lamina samples macerated for the 1948 experiment were ground with purified quartz sand in 50-ml pyrex beakers with small glass pestles, an extracting reagent being added before grinding. For subsequent experiments samples of soluble iron or chlorophyll determination were macerated in the presence of ground glass or carborundum by a mechanically-driven all-glass homogeniser; samples for soluble K, P and Ca estimation were macerated in the "N.I.R.D." homogeniser, manufactured by Nelco Ltd.

iii. Estimation of chlorophyll. Each sample was macerated with 85% (V/V) acetone in the presence of about 5 mg of calcium carbonate and the extract made up to 50 ml with the extractant. Colorimetric measurement was carried out with a Spekker Absorptiometer using red filters. The chlorophyll calibration curve was obtained by adapting the methods described by Comar and others[2) 3) 4)].

iv. Determination of soluble K, P and Ca status. The term "soluble" is here used to describe that fraction of potassium phosphorus or calcium present in the lamina which was extracted by Morgan's reagent[14)]. The term is used without reference to any specific

metabolic activity. In the 1948 experiment 1 g of lamina was macerated with 20 ml of Morgan's reagent in the presence of 0.2 g of carbon. In later experiments 3 g of lamina were macerated with 30 ml of Morgan's reagent in the presence of 0.6 g of carbon. All filtrates were colourless. The methods of chemical estimation were those described by Nicholas [14]).

v. Soluble iron. The terms "soluble", "active" or "available" iron were first used by Oserkowsky [17]) to designate that fraction of the iron present in leaves which he considered to be active in chlorophyll formation. He found the active fraction to be more abundant in the green leaves than in the chlorotic ones, although the reverse may be true for the total amount of iron in the leaves.

The methods used to determine soluble iron were based on preliminary results obtained in 1947 when numerous reagents were used to extract iron from the petioles of chlorotic (iron deficient) and green leaves of tomato, clover and sugar beet. Two reagents consistently extracted differential amounts from green and chlorotic leaves: 0.1 *N* HCl saturated with ether and 1.72% glycerophosphoric acid. The first extracted more ferrous and less ferric iron from the green than from the chlorotic leaves while the glycerophosphoric acid extracted little, if any ferric iron either from green or chlorotic leaves.

The hydrochloric acid and ether reagents used were purified by distillation. The iron was removed from the glycerophosphoric acid either as thiocyanate in a 1 : 1 mixture of amyl alcohol and amyl acetate or as hydroxyquinolate in chloroform. All extracting reagents were adjusted to a pH of 1.0.

The laminae were washed in 0.02 *N* HCl and dried between sheets of blotting paper before maceration. Extracts for the 1948 experiment were prepared from 2 g of lamina and 20 ml of extracting reagent; these proportions were later changed to 1 or 2 g of lamina per 30 ml of reagent. The final extracts were evaporated, wet digested and the iron content estimated with α,α-dipyridyl.

vi. Estimation of total nutrient concentration. 20 ml of nitric and 2 ml of perchloric acids per gram of dry matter were used in the wet digestion; no sulphuric acid was added. The final digests were analysed for iron, potassium, phosphorus and calcium using methods previously described [9]). The values obtained for potassium, phosphorus and calcium in leaf material from the same treatment in different blocks were reasonably concordant but for iron the agreement between duplicate plots was generally less satisfactory.

4. *Presentation of results*

Most of the experiments to be described were based on a factorial design; it is therefore possible to present the data as condensed mean tables which show clearly the overall and interaction effects. Standard errors are included within each table. It has only been possible to summarize a few of the more important results but the complete numerical data are available for consul-

Long Ashton Research Station. In the account of the results an attempt has been made to describe the effects general to all the experiments. Where effects were noted only in one particular experiment the latter is then referred to.

RESULTS

1. *Visual observations*

In all the experiments iron deficiency chlorosis appeared a month after planting in the Fe_1K_1 plants; it appeared first in the C_2 and in the P_2 and P_3 treatments. (Plate I).

In the 1948 experiment chlorosis appeared, but with diminished severity as the iron level increased, in the following plants: Fe_1K_1-C_1 and C_2, Fe_1K_2-C_1 and C_2, $Fe_1K_3C_2$, Fe_2K_1-C_1 and C_2, Fe_2K_2-C_1 and C_2, $Fe_2K_3C_2$, $Fe_3K_1C_2$, $Fe_3K_2C_2$. The young leaves of the $Fe_1K_3C_1$, $Fe_1K_4C_1$, Fe_2K_3-C_1 and C_2, Fe_2K_4-C_1 and C_2, $Fe_3K_3C_2$ plants were green and the old leaves relatively paler, in contrast to the corresponding K_1 plants in which the apical leaves remained chlorotic. This change in the relative distribution of green colour ("Fe/K visual response", Plate II) previously noted by Hewitt and Bolle-Jones [9]) was thus confirmed. The type of iron deficiency chlorosis observed was similar for both C_1 and C_2 plants; the symptoms of the C_2 plants were generally more severe than the corresponding C_1.

In the 1949 experiments the "Fe/K visual response" was apparent in the $Fe_1K_3P_2$ and $Fe_1K_3P_3$ plants at the beginning of the season (Plate II) but later these plants became apically chlorotic. Iron deficiency chlorosis was confined to the Fe_1 plants and was observed in the P_2, P_3 and K_1P_1 treatments during the experiment. Increased amounts of phosphate consistently increased the severity of the chlorosis at the Fe_1 level (Plate III).

Potassium deficiency symptoms were observed three to five weeks after planting in the Fe_1K_1 plants in the 1948 experiment and in the $Fe_1K_1P_3$ plants of the 1949–1951 experiments; symptoms did not appear in the corresponding high iron plants until a week later (Plate IV). At any one potassium level the appearance of potassium deficiency was delayed by an increase in the iron level of the nutrient (Plate IV). Potassium deficiency symptoms, which were apparent at the K_1 and K_2 levels only, were more severe in the C_2 plants and at the higher phosphate levels. Potassium deficiency at low iron levels was accompanied by continued apical growth and a marked defoliation of the lower region of the plant. At the higher iron levels,

however, the symptoms were more drastic; they spread to the youngest leaves and caused the collapse and withering of the plant (Plate V).

Thus the effect of iron in delaying the inception of potassium deficiency and increasing its ultimate severity (Hewitt and Bolle-Jones[9]) was fully confirmed.

Where phosphate was applied at the lowest level phosphorus deficiency symptoms were observed, six weeks after planting, in the Fe_3K_2 and Fe_3K_3 plants. These symptoms were more severe at the high iron, high potassium and low phosphorus levels and were observed in the following treatments (of the 1949 experiment):

$Fe_1K_2P_1$, $Fe_1K_3P_1$, $Fe_2K_2P_1$, $Fe_2K_3P_1$, $Fe_2K_3P_2$, $Fe_3K_1P_1$, $Fe_3K_2P_1$, $Fe_3K_3P_1$, $Fe_3K_3P_2$.

It was interesting to note that the $Fe_3K_1P_1$ plants developed phosphorus deficiency symptoms while the $Fe_3K_1P_3$ and $Fe_1K_1P_1$ plants showed potassium deficiency symptoms.

Symptoms attributed to combined effects of calcium deficiency and low phosphorus status were observed fifteen weeks after planting in the 1948 experiment and usually 8–10 weeks after planting in the subsequent experiments. The laminae became reduced in size, the margins became wavy and inrolled towards the dorsal surface and a basal chlorosis appeared (Plate VI). The straplike leaflets withered from the tip downwards and turned a bright yellow; death of the leaflets followed but the stem growing point continued upwards producing elongated internodes and leaflets progressively reduced in size. Brown lesions appeared along the stem and the growing point was finally killed. In contrast to the rapid formation and pre-mature death of the young laminae, the old laminae retained their green colour and remained attached to the stem for long periods. In the 1948 experiment these symptoms occurred in both C_1 and C_2 series in the K_2, K_3 and K_4 plants at the Fe_3 and Fe_4 levels. The symptoms increased in severity with either iron or potassium level but were less severe where calcium carbonate had been added.

In the 1949 experiment these symptoms were first apparent in the $Fe_1K_3P_1$, $Fe_2K_3P_1$ and Fe_3K_3-P_1, P_2 and P_3 plants and hence appeared to be caused by the high potassium level (Plate VII). They were observed also in the $Fe_2K_2P_1$, Fe_2K_3-P_2 and P_3, and $Fe_3K_2P_1$ plants at a later date. In general the incidence and severity

of these calcium-phosphorus deficiency symptoms increased with iron or potassium (Plate VII) or decreased phosphate, supplies.

In the 1951 experiment it was noted that the $Fe_1K_1P_1$ and $Fe_1K_1P_3$ plants supplied with oxalic acid showed a more severe iron deficiency chlorosis than those not supplied with acid; malic acid had a similar but much milder effect. The addition of citric acid did not increase the severity of these symptoms but neither did it alleviate their severity. An effect of organic acid addition on the occurrence or severity of potassium deficiency symptoms was not noted.

2. *The occurrence of tuber necrosis*

A close examination of the tubers produced by plants exhibiting calcium deficiency — low phosphorus effects in the shoot, revealed that small, malformed, corky tubers (described as calcium deficient by Hewitt [8]), and Wallace [36])) occurred sporadically in the higher potassium treatments. In addition two distinct types of tuber necrosis were observed and are described below, with special reference to the 1949 experiment.

(i) External necrosis. This originated at the "rose" end of the tuber. It was visible externally as a dry, brown, partially rotted region situated mainly in the cortex; sometimes it extended into and became continuous with a medullary necrosis, described below. No pathogen was detected and the occurrence did not exceed 15%. The external necrosis appeared in the K_3 treatments.

(ii) Medullary necrosis (Plate VIII). In numerous instances the central or medulla region of tubers, which appeared to be externally normal, was completely rotted. This breakdown originated in the central region of the tuber and was not necessarily connected to the "rose" end exterior. It began as numerous brown necrotic spots, close together in the middle of the medulla. These were small groups of cells the contents of which became granular and orange brown in colour. These cells later became disorganised, adjacent regions coalesced and ultimately broke down to form a brown cavity, later turning black. Medullary necrosis was always found in malformed calcium deficient tubers. The symptoms were not those of "Spraing", "Internal rust spot" or the "Net necrosis" reviewed by Quanjer [19]). The application of potassium increased while that of phosphorus decreased the occurrence of medullary necrosis;

Plate I. Potato plant given $Fe_1K_1P_3$ nutrient. Note intense apical chlorosis and necrotic veining of younger laminae and potassium deficiency in the old.

Plate II. Effect of potassium on iron deficiency. *Left:* Low Fe and high K nutrient. *Right:* Low Fe and low K nutrient. *Note:* apical iron deficiency chlorosis and severe potassium deficiency symptoms in low K plant; dark green young leaves and paler midstem leaves of high K plant.

Plate III. The curative effect of *decreased* phosphorus level on iron deficiency chlorosis. $Fe_1K_1P_3$ (*left*) : $Fe_1K_1P_1$ (*right*). Note dark green vigorous growth of $Fe_1K_1P_1$ plant and acute apical chlorosis in $Fe_1K_1P_3$ plant.

Plate IV. The delaying effect of iron level on the inception of potassium deficiency in the early stages of the experiment. $Fe_1K_1P_3$(*left*) : $Fe_3K_1P_3$ (*right*). Note absence of potassium deficiency symptoms in Fe_3 plant.

Plate V. Close up of the apical region of $Fe_3K_1P_3$ plant to show extreme severity of the potassium deficiency effects during the later stages of the 1949 experiment. Note reduction in size, malformation and necrotic margins of young laminae.

Plate VI. Young leaves showing induced calcium deficiency effects associated with low phosphorus status. Note basal chlorosis, inrolling of margins, gradual attenuation in length and reduction in size as symptoms become more severe.

Plate VII. Effects of potassium in inducing calcium deficiency symptoms associated with low posphorus status in young laminae. $Fe_1K_1P_1$ plant (*left*) : $Fe_1K_3P_1$ plant (*right*). *Note :* slight potassium deficiency in the old laminae of the K_1 plant ; calcium deficiency in the young and phosphorus deficiency in the old leaves of the K_3 plant ; changed habit of K_3 plant.

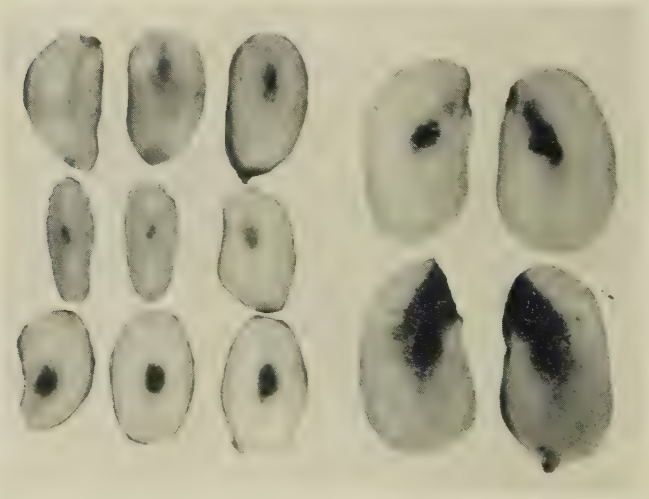

Plate VIII. *Left :* stages in development of medullary necrosis in tubers. *Right :* medullary necrosis becoming continuous with external necrosis.

these effects were highly significant (Table II). The effect of iron supply was complex; potassium supply was the determining factor.

TABLE II

Incidence of medullary necrosis. Means obtained by an angular transformation of the percentage values to illustrate the main effects and interactions. 1949 experiment

Treatment level	K_1	K_2	K_3	P_1	P_2	P_3	Mean
					(± 0.86)		(± 0.50)
Fe_1	5.6	8.7	24.4	19.2	11.4	8.2	12.9
Fe_2	11.1	13.0	26.4	21.8	17.4	13.1	17.5
Fe_3	8.5	13.4	22.9	15.8	16.97	12.1	14.9
P_1	9.8	13.9	33.1	—	—	—	18.9
P_2	9.5	13.6	22.8			-	15.3
P_3	5.9	7.7	19.8	—		-	11.2
Mean	8.4	11.7	25.2	—	—	—	15.1
		(± 0.50)					

3. *Yield data*

In general the effects of potassium and phosphate addition were to increase the dry weight yield of shoots, roots and tubers while calcium carbonate decreased both shoot and tuber yields. The ad-

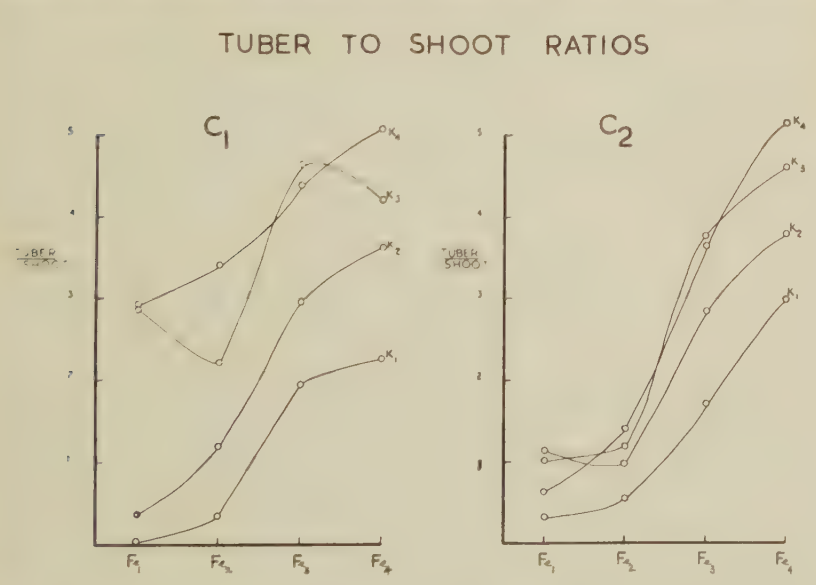

Fig. 1. Variation of dry weight ratio of tuber to shoot with iron level. 1948 experiment.

dition of iron reduced the dry weight of both shoots and roots but increased that of the tubers. This effect was reflected in the striking, and perhaps economically important, increase in the ratio of tuber to shoot which was obtained on the application of iron (Fig. 1).

The yield of tubers obtained in the 1948 experiment furnished a clear instance of the interrelationship which existed between iron and potassium (Table III). Thus addition of iron at the K_1 level caused a 11-fold increase in yield but had relatively little effect at the K_4 level; the response to potassium at the Fe_1 level was 27-fold as compared with a 2–3 fold increase in yield at the Fe_4 level.

TABLE III

Dry weight of tubers (grams per 4 plants). Mean values. 1948 experiment							
Treatment level	Fe_1	Fe_2	Fe_3	Fe_4	C_1	C_2	Mean
K_1	13	54	151	144	90	91	90
K_2	73	172	314	289	224	199	212
		(± 29.8)			(± 21.0)		(± 14.9)
K_3	295	223	527	411	449	279	364
K_4	356	482	532	390	623	258	440
C_1	292	332	415	347			347
		(± 21.0)					(± 10.5)
C_2	76	134	347	270			207
Mean	184	233	381	308	347	207	277
		(± 14.9)			(± 10.5)		

Tubers, although small in weight and few in number, were present in the 1950 experiment when Blocks 7 and 8 were harvested; the yield rose sharply by the time Blocks 3 and 4 were harvested. The total dry weight of the complete plants (Table IV) increased with

TABLE IV

Total dry weight of complete plants. Blocks 3, 4, 7 and 8. (grams per 4 plants). Mean values. 1950 experiment														
Month	S.E. horizontal comparison	Treatment level												S.E. vertical comparison
		Fe_1		Fe_3		K_1		K_3		P_1		P_3		
		K_1	K_3	K_1	K_3	P_1	P_3	P_1	P_3	Fe_1	Fe_3	Fe_1	Fe_3	
June Blocks 7 & 8	± 10.4	82	96	90	88	70	100	80	103	85	66	90	113	± 12.6
	± 7.3	89		89		85		92		76		102		± 8.9
July Blocks 3 & 4	± 10.4	161	266	165	229	154	172	196	300	206	144	222	250	± 12.6
	± 7.3	214		197		163		248		175		236		± 8.9

either potassium or phosphate application. The addition of iron decreased the yield at the P_1 but had no significant effect at the P_3 level.

The provision of organic acid supplements in the 1951 experiment did not enhance the total dry weight yield per plant. Citric acid did not have a detrimental effect whereas oxalic acid did and markedly reduced growth in the $Fe_1K_1P_3$ plants. Malic acid was equally deleterious when given to the same plants but where iron deficiency was not so severe or apparent (as in the $Fe_1K_1P_1$ and $Fe_1K_3P_3$ plants) it did not severely retard growth.

4. *Chemical data*

i. Chlorophyll concentration of the laminae (Table V). The application of iron increased the chlorophyll concentration of young and old laminae; this increase was greater at the lower potassium levels for the young laminae and at the higher phosphate levels for the old laminae.

TABLE V

Chlorophyll concentration of laminae expressed as mg per gram of dry matter
Means of all values for June, July and August 1949

Treatment level	Young laminae							Old laminae						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	(± 0.23)			(± 0.23)			(± 0.14)	(± 0.28)			(± 0.28)			(± 0.16)
K_1	6.6	9.0	8.9	9.2	8.3	7.0	8.2	5.0	6.0	6.8	7.1	5.6	5.1	5.9
K_2	7.7	9.5	9.6	9.1	9.2	8.5	8.9	6.4	8.0	9.0	8.7	7.4	7.3	7.8
K_3	8.1	8.7	8.1	7.5	9.0	8.4	8.3	6.8	8.6	8.9	8.1	8.2	8.1	8.1
P_1	7.4	9.2	9.2	—	—	—	8.6	7.1	8.0	8.7	—	—	—	8.0
P_2	7.7	9.8	9.0	—	—	—	8.8	5.7	7.2	8.2	—	—	—	7.1
P_3	7.2	8.2	8.4	—	—	—	8.0	5.4	7.4	7.8	—	—	—	6.8
Mean	7.4	9.1	8.9	—	—	—	8.5	6.1	7.5	8.2	—	—	—	7.3
	(± 0.14)							(± 0.16)						

The addition of potassium increased the chlorophyll concentration of the old laminae at all iron levels and of the young laminae at the Fe_1 level, but caused a decrease at the higher iron levels. The response to potassium, in the 1948 experiment, was more pronounced for the C_1 than for the C_2 plants for both young and old laminae. In the subsequent experiments addition of potassium decreased the chlorophyll concentration of the young laminae at the P_1 level but increased it at the P_2 and especially at the P_3 levels. Increased potassium

supply generally increased the chlorophyll content of the old laminae at all phosphate levels.

Calcium carbonate addition decreased the chlorophyll concentration of both young and old laminae and particularly so for the low iron treatments.

The application of phosphate reduced the chlorophyll concentration of both young and old laminae; this reduction was most marked at the K_1 level and least apparent at the K_3 level. The depressive influence of phosphate was greatest at the Fe_1 level in the old laminae.

It was noted, for the 1951 experiment, that organic acid addition did not generally increase the chlorophyll content of the laminae; in fact, oxalic acid addition markedly reduced the chlorophyll concentration, as also did malic acid to some extent.

ii. Titratable acidity. Representative values obtained for titratable acidity are summarised in Table VI. Increased potassium level decreased the acidity of the young laminae but either had little effect or increased that of the old laminae. Thus the increase which occurred in the chlorophyll concentration of the young laminae of the Fe_1P_3 plants when the potassium supply was increased was accompanied by a decrease in the titratable acidity. Phosphorus addition invariably increased the acidity of both young and old laminae. The influence of either iron or organic acid supplements was not consistent.

TABLE VI

Titratable acidity (Volume, ml of 0.01 *N* NaOH required to bring extract to pH 9.5)

Young laminae	1950	1951	Young laminae 1951							
$Fe_1K_1P_1$	8.2	7.6	Control	$Fe_1K_1P_1$	6.9	$Fe_1K_1P_3$	14.0	$Fe_1K_3P_3$	8.2
$Fe_1K_1P_3$	12.0	13.8	Malic	$Fe_1K_1P_1$	5.8	$Fe_1K_1P_3$	13.0	$Fe_1K_3P_3$	10.7
$Fe_1K_3P_3$	10.5	9.7	Citric	$Fe_1K_1P_1$	6.7	$Fe_1K_1P_3$	12.2	$Fe_1K_3P_3$	11.9
$Fe_3K_3P_3$	11.0	11.8	Oxalic	$Fe_1K_1P_1$	7.2	$Fe_1K_1P_3$	13.4	$Fe_1K_3P_3$	11.8
Old laminae	1950		Old laminae 1951							
$Fe_1K_1P_1$	6.0	—	Control	$Fe_1K_1P_1$	5.2	$Fe_1K_1P_3$	11.6	$Fe_1K_3P_3$	13.2
$Fe_1K_1P_3$	7.2	—	Malic	$Fe_1K_1P_1$	3.8	$Fe_1K_1P_3$	14.8	$Fe_1K_3P_3$	16.8
$Fe_1K_3P_3$	10.6	—	Citric	$Fe_1K_1P_1$	3.9	$Fe_1K_1P_3$	10.9	$Fe_1K_3P_3$	9.9
$Fe_3K_3P_3$	11.8	—	Oxalic	$Fe_1K_1P_1$	—	$Fe_1K_1P_3$	9.1	$Fe_1K_3P_3$	9.5

1950 extracts prepared by extracting 5 g fresh laminae with 60 ml of 85% alcohol; 1951 extracts prepared by taking 3 ml of sap expressed from fresh laminae and diluting with water to 25 ml before titration.

Samples of juice expressed from frozen laminae of plants grown in the 1951 experiment were examined chromatographically for organic acid constituents by Mr. A. H. Williams. None of the commoner acids was readily detected in the juice. One "unknown" acid only showed variation with mineral treatment; the amount of this acid present did not vary consistently as a result of the organic acids supplied in the nutrients. This "unknown" acid possessed an R_F value similar to malic acid when the chromatograms were run in an acid solvent (butanol-formic acid) but similar to lactic acid when run in an alkaline solvent (ethanol-ammonia). The "unknown" acid was always found in least concentration in juice taken from the $Fe_1K_1P_3$ laminae and in highest concentration in the $Fe_1K_3P_3$ laminae.

Apparently the organic acids fed to the plants either became localised in the roots, tubers or stems or were metabolised completely. In one instance only was there any indication that the plants had been fed with organic acids when a large genuine malic acid spot was obtained in the old laminae taken from $Fe_1K_3P_3$ plants fed with malic acid.

iii. Inorganic and organic phosphorus in laminae (Table VII). Fresh laminae were macerated with ice-

TABLE VII

Inorganic and organic phosphorus concentrations of young laminae (expressed as mg P per gram dry matter). Mean values. 1951 experiment

Treatment	1st sampling					2nd sampling				
	Inorg. P	Org. P	Inorg. Total	Org. Total	Inorg. Org.	Inorg. P	Org. P	Inorg. Total	Org. Total	Inorg. Org.
$Fe_1K_1P_3$	7.11	1.32	.79	.21	3.72	6.23	6.23	.50	.50	1.00
$Fe_1K_2P_3$	5.54	1.56	.78	.22	3.55	3.99	5.88	.40	.60	0.68
$Fe_1K_3P_3$	1.90	2.05	.48	.52	0.93	2.12	4.59	.32	.68	0.46
$Fe_2K_2P_3$	4.28	2.00	.68	.32	2.14	5.16	4.26	.55	.45	1.21
$Fe_3K_2P_3$	3.72	3.02	.55	.45	1.23	3.33	4.19	.44	.56	0.80

cold 10% perchloric acid. The estimation of inorganic phosphorus in the supernatant liquid after centrifuging was based on procedures described by Weil-Malherbe and Green [38]). The amount of organic phosphorus in the laminae was obtained by subtracting the inorganic phosphorus value from the total phosphorus value determined by the usual wet digestion procedure and subsequent colorimetric estimation.

The concentration of inorganic phosphorus and its proportion of

the total phosphorus in the lamina, decreased as the potassium level of the nutrient increased. Potassium level did not usually affect the concentration of organic phosphorus present but increased its proportion of the total (*i.e.* organic P/total P). Increased potassium supplies markedly decreased the ratio value for inorganic/organic phosphorus found in the laminae.

Increased iron level generally increased the concentration and proportion of inorganic phosphorus present.

iv. Morgan's-soluble K, P and Ca (Table VIII). Increased iron supply decreased the soluble potassium concentration of the young laminae at the K_1 level but increased the potassium concentration of the old laminae at all K levels. The addition of calcium carbonate tended to increase the potassium concentration of the young laminae but significantly reduced that of the old laminae. Phosphate addition caused an overall decrease in the potassium concentration of the old laminae.

TABLE VIII

% Morgan's-soluble potassium, phosphorus and calcium in laminae, expressed on dry weight basis. Overall mean values. July sampling 1949

Treatment level	Young laminae			Old laminae		
	% K	% P	% Ca	% K	% P	% Ca
	(± .08)	(± .017)	(± .054)	(± .08)	(± .008)	(± .07)
Fe_1	2.1	.20	.50	1.5	.19	2.0
Fe_2	1.7	.13	.28	1.7	.13	1.7
Fe_3	1.8	.12	.55	2.0	.12	1.8
	(± .08)	(± .017)	(± .054)	(± .08)	(± .008)	(± .07)
K_1	1.3	.20	.60	0.4	.19	2.1
K_2	1.5	.15	.54	1.0	.16	2.4
K_3	2.8	.11	.19	3.8	.10	1.1
	(± .08)	(± .017)	(± .054)	(± .08)	(± .008)	(± .07)
P_1	1.8	.04	.44	2.0	.03	1.5
P_2	1.9	.12	.42	1.6	.07	2.0
P_3	1.9	.30	.47	1.5	.34	2.0

The addition of iron or potassium decreased the soluble phosphorus concentration of the young laminae; this also applied to the old laminae for the 1949 and subsequent experiments. The effects of both iron and potassium were most marked at the P_3 level. The addition of calcium carbonate decreased the phosphorus concentration of the old laminae.

The effect of iron level on soluble calcium concentration was not consistent. Increased potassium supply reduced the soluble calcium concentration of young and old laminae. The application of calcium carbonate or phosphate produced an overall increase in the soluble calcium concentration of young and old laminae.

v. Soluble iron. Of the two types of extractant tested, hydrochloric acid saturated with ether appeared to give values better related to the visual appearance of the plants, and more consistent results, than glycerophosphoric acid. In general both reagents extracted more soluble iron from the old laminae of plants supplied with the higher levels of potassium; this response to potassium was sometimes significant when glycerophosphoric acid was used but always so when the other reagent was employed (Table IX).

TABLE IX

Ppm soluble iron, extracted by 0.1 *N* HCl + ether, in the dry matter of laminae. Mean values. August sampling 1949

Treatment level	Young laminae							Old laminae						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	(± 14.0)			(± 14.0)			(± 8.1)	(± 9.6)			(± 9.6)			(± 5.5)
K_1	27	32	60	42	46	32	40	44	45	47	48	44	44	45
K_2	33	26	44	51	29	23	34	54	59	65	70	74	34	59
K_3	49	50	73	86	61	26	58	74	91	82	109	108	30	82
P_1	33	53	93	—	—	—	60	74	72	82	—	—	—	76
P_2	43	30	63	—	—	—	45	59	82	85	—	—	—	75
P_3	34	25	22	—	—	—	27	40	42	27	—	—	—	36
Mean	37	36	59	—	—	—	44	57	65	65	—	—	—	62
	(± 8.1)							(± 5.5)						

vi. Ash analysis of laminae. Iron concentration (Table X). Addition of iron usually, but not always, produced an overall increase in the iron concentration of both young and old laminae; this increase was largest at the lower P levels. Potassium addition increased the iron concentration of both young and old laminae. The addition of calcium carbonate reduced the iron concentration of young and old laminae; this depressive effect was usually greater in the old than in the young laminae. Phosphate addition markedly decreased the iron concentration of the old laminae and to a lesser extent, of the young laminae; this effect was usually strongest at the high iron levels.

Potassium concentration (Table X). The trends obtained for the total potassium concentration were similar to those obtained for the soluble potassium. Thus iron addition decreased the potassium concentration at the K_1 level and increased it at the K_3 level for the young laminae and increased it at all K levels in the old laminae.

TABLE X

Ppm iron and % potassium in dry matter of laminae. Mean values. 1949 experiment

Treatment level	Iron, ppm							Potassium, %						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	Young laminae (July)							Young laminae (July)						
	(± 28.2)			(± 28.2)			(± 16.3)	(± 0.19)			(± 0.19)			(± 0.11)
K_1	153	199	177	222	170	138	176	2.6	2.2	1.9	1.6	2.1	2.3	2.0
K_2	174	211	158	200	177	165	181	2.3	2.4	2.1	2.0	2.3	2.5	2.3
K_3	192	225	214	237	210	184	210	3.0	3.9	4.3	3.7	3.9	3.6	3.7
P_1	163	224	272	—	—	—	220	2.4	2.6	2.4	—	—	—	2.4
P_2	205	200	151	—	—	—	185	2.8	2.7	2.7	—	—	—	2.8
P_3	150	210	126	—	—	—	162	2.7	3.2	2.5	—	—	—	2.8
Mean	173	212	183	—	—	—	189	2.6	2.8	2.5	—	—	—	2.7
	(± 16.3)							(± 0.11)						
	Old laminae (August)							Old laminae (July)						
	(± 112)			(± 112)			(± 64.4)	(± 0.10)			(± 0.10)			(± 0.06)
K_1	332	258	529	703	296	119	373	0.4	0.8	0.7	0.7	0.5	0.7	0.6
K_2	343	493	624	719	587	153	486	1.1	1.4	1.7	1.9	1.2	1.2	1.4
K_3	438	640	648	985	572	168	575	4.4	4.9	5.4	5.7	4.8	4.3	4.9
P_1	717	770	920	—	—	—	802	2.4	2.6	3.2	—	—	—	2.8
P_2	265	480	710	—	—	—	485	1.7	2.3	2.6	—	—	—	2.2
P_3	130	140	170	—	—	—	147	1.8	2.3	2.0	—	—	—	2.0
Mean	371	464	600	—	—	—	478	2.0	2.4	2.6	—	—	—	2.3
	(± 64.4)							(± 0.06)						

Increased supplies of potassium were more effective in increasing the potassium concentration of the laminae in the C_2 than in the C_1 plants. The addition of phosphate had no consistent effect on the young laminae but decreased the potassium concentration of the old-especially at the Fe_3 and K_3 levels (Table X).

Phosphorus concentration (Table XI). Increased supplies of either iron or potassium or the addition of calcium carbonate decreased the phosphorus concentration of both young and old laminae.

Calcium concentration (Table XI). Iron addition decreased the calcium concentration of the old laminae; its effect on the young laminae was not consistent. Increased potassium supply usually produced an overall non-linear decrease in the calcium concentration of young and old laminae. Calcium carbonate addition increased the calcium concentration of young and old laminae particularly those of the low iron plants. Increased phosphate supply resulted in an overall increase in the calcium concentration of the old laminae.

vii. Ash analysis of tubers. The addition of iron increased and that of calcium carbonate decreased the iron concentration of the tubers (Table XII). The application of potassium

TABLE XI

% Phosphorus and % calcium in dry matter of laminae. Mean values for July sampling 1949

Treatment level	Phosphorus, %							Calcium, %						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	Young laminae							Young laminae						
	(± .021)			(± .021)			(± .012)	(± 0.07)			(± 0.07)			(± 0.04)
K_1	.66	.50	.45	.30	.49	.83	.54	1.4	1.2	1.6	1.4	1.3	1.5	1.4
K_2	.54	.45	.41	.27	.42	.72	.47	1.2	1.1	1.2	1.3	1.0	1.1	1.2
K_3	.47	.37	.35	.24	.47	.58	.40	0.6	0.6	0.8	0.6	0.7	0.7	0.7
P_1	.32	.25	.24	—	—	—	.27	1.0	1.0	1.4	—	—	—	1.1
P_2	.52	.44	.32	—	—	—	.42	1.1	0.8	1.0	—	—	—	1.0
P_3	.83	.63	.66	—	—	—	.71	1.1	1.0	1.2	—	—	—	1.1
Mean	.56	.44	.40	—	—	—	.47	1.1	0.9	1.2	—	—	—	1.1
	(± .012)							(± 0.04)						
	Old laminae							Old laminae						
	(± .019)			(± .019)			(± .011)	(± 0.10)			(± 0.10)			(± 0.06)
K_1	.50	.52	.32	.15	.29	.90	.45	3.6	2.9	2.9	3.0	3.5	2.9	3.1
K_2	.47	.38	.31	.13	.22	.80	.38	4.2	3.5	3.2	2.9	4.1	3.8	3.6
K_3	.29	.26	.24	.13	.19	.48	.26	2.3	1.9	1.8	1.7	2.2	2.2	2.0
P_1	.16	.15	.09	—	—	—	.14	3.1	2.2	2.3	—	—	—	2.6
P_2	.27	.26	.18	—	—	—	.23	3.7	3.3	2.7	—	—	—	3.2
P_3	.83	.75	.61	—	—	—	.73	3.3	2.8	2.9	—	—	—	3.0
Mean	.42	.38	.29	—	—	—	.37	3.4	2.8	2.6	—	—	—	2.9
	(± .011)							(± 0.06)						

TABLE XII

Ppm iron (total concentration) in dry matter of tubers. Mean values. 1948 experiment

Treatment level	Fe_1	Fe_2	Fe_3	Fe_4	C_1	C_2	Mean
K_1	10.5	8.6	16.3	23.5	17.1	12.4	14.7
K_2	8.1	9.6	13.6	21.4	14.7	11.7	13.2
		(± 2.21)			(± 1.56)		(± 1.11)
K_3	10.4	8.2	21.3	28.0	20.5	13.3	16.9
K_4	8.3	10.4	21.9	32.4	19.4	17.1	18.2
C_1	9.9	11.1	22.1	28.6	—	—	17.9
		(± 1.56)					(± 0.78)
C_2	8.7	7.3	14.4	24.0	—	—	13.6
Mean	9.3	9.2	18.3	26.3	17.9	13.6	15.8
		(± 1.11)			(± 0.78)		

TABLE XIII

Total iron, potassium and phosphorus concentrations of roots. Mean values for 1949 experiment

Treatment level	Ppm Fe in dry matter						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	(± 36.2)			(± 36.2)			(± 20.9)
K_1	97	139	517	371	258	125	251
K_2	140	129	685	514	250	172	312
K_3	94	188	941	490	397	337	408
P_1	152	189	1033	—	—	—	458
P_2	101	157	647	—	—	—	302
P_3	78	109	446	—	—	—	211
Mean	111	152	709	—	—	—	324
	(± 20.9)						

Treatment level	% K in dry matter						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	(± 0.146)			(± 0.146)			(± 0.084)
K_1	0.56	0.40	0.29	0.27	0.36	0.63	0.42
K_2	0.59	0.60	0.55	0.66	0.43	0.64	0.58
K_3	2.46	3.37	1.74	2.50	2.05	3.01	2.52
P_1	1.29	1.35	0.80	—	—	—	1.15
P_2	1.23	1.11	0.48	—	—	—	0.94
P_3	1.08	1.90	1.30	—	—	—	1.43
Mean	1.20	1.45	0.86	—	—	—	3.52
	(± 0.084)						

Treatment level	% P in dry matter						
	Fe_1	Fe_2	Fe_3	P_1	P_2	P_3	Mean
	(± .042)			(± .042)			(± .024)
K_1	.51	.45	.20	.10	.19	.87	.39
K_2	.32	.38	.20	.09	.14	.66	.30
K_3	.37	.27	.16	.08	.11	.62	.27
P_1	.09	.09	.09	—	—	—	.09
P_2	.18	.13	.13	—	—	—	.15
P_3	.93	.89	.33	—	—	—	.72
Mean	.40	.37	.18	—	—	—	.32
	(± .024)						

increased the iron concentration of the tubers at the high iron levels. Generally the variations in iron concentration according to treatment were more consistent in the tubers than in the laminae.

Although not presented here, it was found that the application of iron decreased the potassium concentration at all potassium levels while calcium carbonate usually increased it; the addition of iron or

potassium decreased botht he calcium and phosphorus concentrations tound in the tubers.

viii. Ash analysis of roots (Table XIII). The nutrient concentrations within the roots were more susceptible to the effect of treatment than were either the laminae or the tubers.

The iron concentration of the roots increased with iron and potassium levels but decreased with phosphate addition.

Increased iron supply linearly decreased the potassium concentration of the roots in the K_1 treatments and gave a non-linear decrease in the K_3 treatments. Phosphate addition decreased the potassium concentration at the Fe_1 level but increased it at higher iron levels.

Increased iron or potassium supplies gave an overall decrease in the phosphorus concentration of the roots. Iron application had no effect on phosphorus concentration at the P_1 level but caused a large decrease at the P_3 level.

5. *Total nutrient content of the potato plant* (1950 experiment)

The results described in this section and sections 6 and 7 refer specifically to the 1950 experiment and deal only with iron, potassium and phosphorus. Although similar data were obtained for calcium and are available for consultation at Long Ashton Research Station, they have been omitted from this paper mainly on the grounds of space and because they do not affect the main conclusions drawn.

The results given in Table XIV were obtained from Blocks 7 and 8

TABLE XIV

Iron content of complete plants and of shoots and tubers; potassium and phosphorus contents of complete plants (Fe expressed as μg; K and P as mg; per 4 plants). Mean values. Blocks 7 and 8. 1950 experiment

	Standard error	Treatment level											
		Fe_1		Fe_3		K_1		K_3		P_1		P_3	
		K_1	K_3	K_1	K_3	P_1	P_3	P_1	P_3	Fe_1	Fe_3	Fe_1	Fe_3
g Fe	± 748	4650	6886	7406	8569	5005	7252	7329	8146	5602	6732	6134	9264
omplete plants	± 529	5868		7998		6128		7738		6167		7699	
g Fe	± 337	4142	5575	4896	6198	4240	4796	5209	6564	5042	4406	4674	6688
oots and tubers	± 238	4858		5547		4519		5886		4724		5681	
g K/	± 151	1223	5436	1117	4940	1018	1322	4530	5846	3098	2450	3562	3606
omplete plants	± 107	3330		3028		1170		5188		2774		3584	
g P/	± 9.9	377	395	286	328	128	536	136	586	156	108	616	506
omplete plants	± 7.0	386		307		332		361		132		561	

which were chosen for illustration as the effect of treatment on yield, although apparent, was not so marked as in Blocks 3 and 4 (Table IV). Consequently any differences in the nutrient content of the plants grown under different treatments were more likely to be attributed to the direct effect of treatment on uptake and accumulation of the nutrient than to a growth effect.

The iron content of the complete plant increased with iron supply particularly at the K_1 and P_3 levels and with potassium supply at the Fe_1 and P_1 levels (Table XIV). If the total iron content of the shoot and tubers only was considered, and the roots excluded, the standard errors obtained were appreciably smaller and the effect of potassium on iron content became more obvious and was significant at the 1.0% level (Table XIV). The addition of iron decreased the iron content of the shoot plus tubers at the P_1 level but increased it at the P_3 level.

The potassium content of the complete plant increased with increased potassium or phosphate supply (Table XIV), iron addition had little effect.

Increased phosphate supply or of potassium, at the Fe_3 and P_3 levels, increased while iron decreased the phosphorus content of the complete plant (Table XIV).

6. *The relative distribution of nutrients within the potato plant* (1950 experiment)

i. I r o n (Fig. 2). Increased iron level increased the proportion of iron found in the roots and tubers. In June (Blocks 7 and 8) added iron generally increased the proportion found in the stems and petioles of the bottom region.

Potassium addition increased, at the June sampling, the proportion of iron found in the stems and petioles of the top region and also in July in the Fe_1P_3 and Fe_3P_1 treatments. Added potassium decreased, in July, the proportion of iron in the lower stems and petioles at the Fe_3P_3 level; this was probably due to tuber formation. Potassium addition markedly increased the proportion of iron found in the tubers and consistently decreased the iron distributed to the roots, at the July sampling. In June there was evidence that at the P_1 level potassium increased the iron found in the roots.

In July, increased phosphate generally decreased the proportion of iron in the roots, except at the Fe_1K_1 level, when it increased.

Phosphate addition usually decreased the proportion of iron found in the tubers except at the Fe_3K_3 level when an increase was

Fig. 2. The relative distribution of iron within the potato plant 40 days (Blocks 7 and 8) and 72 days (Blocks 3 and 4) after planting. The respective levels of Fe, K and P applied are indicated below the lowest histogram; *e.g.* 133 refers to treatment $Fe_1K_3P_3$. 1950 experiment.

obtained. Phosphate had no consistent effect on the proportion of iron in the laminae in June but tended to give an overall increase in July, especially in the upper laminae.

ii. Potassium (Fig. 3). Increased iron supply increased the

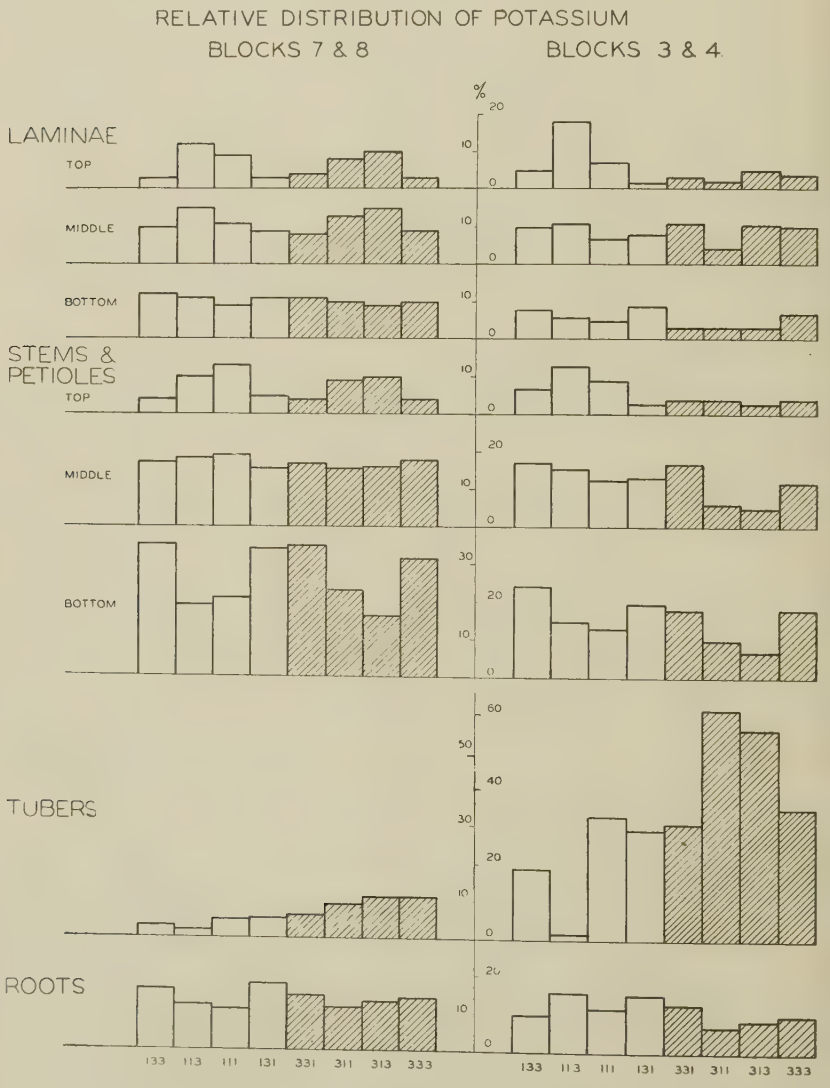

Fig. 3. The relative distribution of potassium within the potato plant. 1950 experiment.

proportion of potassium found in the tubers but usually decreased it, for July, in the roots and top and bottom laminae. A general decrease in the proportion of potassium in the stems and petioles at all levels except the K_3P_1 was obtained on the addition of iron.

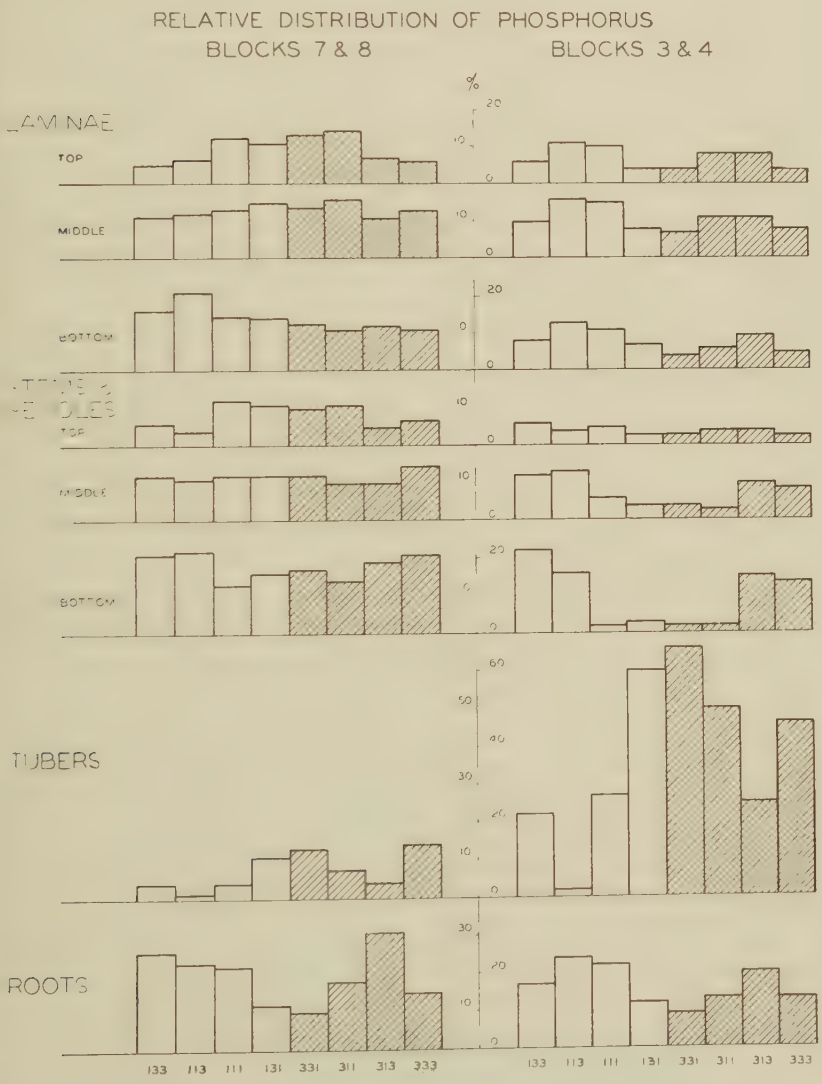

Fig. 4. The relative distribution of phosphorus within the potato plant. 1950 experiment.

Increased potassium supply increased the proportion of potassium found in the roots but decreased it in the tubers, except at the Fe_1P_3 level. Potassium addition decreased the proportion of potassium found in the upper laminae and increased the proportion of potassium in the stems and petioles of the lower regions.

In July increased phosphate supply decreased, (except at the Fe_3K_3 level), the proportion of potassium found in the tubers.

iii. Phosphorus (Fig. 4). The addition of iron generally decreased the proportion of phosphorus found in the roots but increased it in the tubers. Iron addition also decreased the phosphorus content of the laminae and stems and petioles in July.

The addition of potassium usually decreased the phosphorus in the roots but increased it in the tubers. Increased potassium

TABLE XV

Amounts of iron, potassium and phosphorus supplied to potato plants from seed tubers and added nutrients and the percentage of this gross supply absorbed by the plants. Fe, K, and P expressed as mg per 4 plants. 1950 experiment

Treatment	Block number	Amounts supplied from (A) Nutrient			(B) Seed tuber *i.e.* content of seed minus mother tubers			(C) Total supplied (A) + (B)			% of total (C) taken up by plants		
		Fe	K	P	Fe	K	P	Fe	K	P	Fe	K	P
$Fe_1K_1P_1$	3	0.70	1056	422	—1.12	753	50	—0.42	1809	472	—	118	52
	4	0.70	1056	422	—0.42	756	48	+0.28	1812	470	—	95	48
$Fe_1K_3P_1$	3	0.74	17664	442	—2.33	233	42	—1.59	17897	484	—	60	60
	4	0.74	17664	442	—1.17	305	42	—0.43	17969	484	—	60	63
$Fe_1K_1P_3$	3	0.80	1152	4147	+0.41	990	38	+1.21	2142	4185	—	92	32
	4	0.80	1152	4147	+0.12	1198	55	+0.92	2350	4202	—	68	27
$Fe_1K_3P_3$	3	0.78	19200	4320	—0.82	50	—53	—0.04	19250	4267	—	75	34
	4	0.78	19200	4320	—0.97	253	—57	—0.19	19453	4263	—	72	37
$Fe_3K_1P_1$	3	781.2	1008	403	—1.48	705	51	780	1713	454	30	88	37
	4	781.2	1008	403	—1.85	588	47	779	1596	450	27	93	38
$Fe_3K_3P_1$	3	781.2	16128	403	—1.90	285	46	779	16413	449	25	48	44
	4	781.2	16128	403	—2.05	130	44	779	16258	447	23	40	37
$Fe_3K_1P_3$	3	855.6	1104	3974	—0.63	732	18	855	1836	3992	18	104	22
	4	855.6	1104	3974	+0.26	868	1	856	1972	3975	17	86	23
$Fe_3K_3P_3$	3	892.8	18432	4147	—2.10	182	—32	891	18614	4115	41	77	42
	4	892.8	18432	4147	—1.41	467	—36	891	18799	4111	43	64	32

Note: The percentage of iron absorbed from nutrients and seed tubers by the Fe_1 plants cannot be calculated from the data available as the plants obviously obtained large amounts of iron from other sources.

decreased the proportion of phosphorus found in the laminae; this effect was well marked in the top region.

Increased phosphate supply increased the proportion of phosphorus found in the roots but decreased it in the tubers. It also markedly increased the phosphorus found in the stems and petioles of the lower regions for the July sampling. Phosphate addition caused a decrease in the proportion of phosphorus in the upper laminae of the June sampling; in the bottom laminae of both June and July samplings the reverse effect was apparent.

7. *Nutrient absorption as affected by treatment* (1950 experiment). The amounts of iron, potassium and phosphorus supplied to the plant by the seed tuber and by the application of nutrients, are given in Table XV. These results have been calculated from the volume of nutrients applied to the plants before sampling and from the weight and composition of the seed and mother tubers.

i. Changes in the seed tubers (Table XV). The mother tuber contained more iron at the end of the experiment than was initially present in the seed tuber, except for the $Fe_1K_1P_3$ plants which had depleted the iron content of the seed tubers. This increase in the iron content of the tuber was greater in the K_3 treatments which suggested that increased potassium supply caused iron to return from the plant to the mother tuber.

Plants grown at the K_1 and P_1 levels took up more potassium and phosphorus respectively from the seed tubers than those at the K_2 and P_2 levels. A larger withdrawal of potassium took place at the K_1P_3 than at the K_1P_1 level.

The withdrawal of phosphorus from the seed tuber was greater in the K_1 than in the K_2 treatments. The phosphorus content of the mother tuber in the K_2P_3 treatments was greater than the seed tuber. These results suggested that increased potassium supply caused phosphorus to return from the plant to the tuber.

ii. Percentage uptake of total nutrient supplied (Table XV). The percentage of the total iron supplied, from the nutrients and seed tuber, taken up by the plant — *i.e.* the "percentage uptake" — increased with potassium at the Fe_3P_3 level but decreased slightly at the Fe_3P_1. Unfortunately similar data were not available for the Fe_1 treatments as the plants contained more iron than was supplied to them in solution and from the seed tubers.

This implied that the plants took up large quantities of iron from the sand or from some other source.

The "percentage uptake" of potassium decreased with potassium level but increased with phosphate at the K_3 level. The addition of iron decreased the "percentage uptake" of potassium at the P_1 level but had little effect at the P_3 level.

The "percentage uptake" of phosphorus decreased with phosphate level and also with iron, except at the K_3P_3 level when it increased. Increased potassium supply increased the "percentage uptake" of phosphorus.

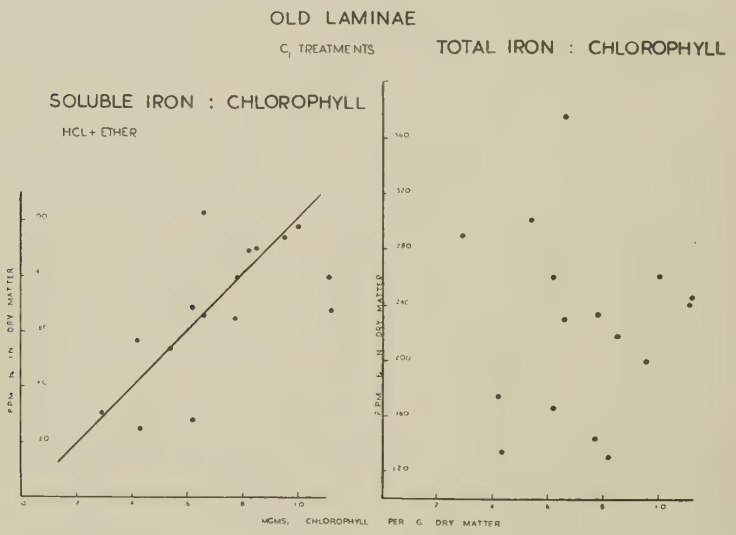

Fig. 5. A comparison of the relationship between (*a*) iron extracted with 0.1 *N* hydrochloric acid saturated with ether, and chlorophyll; (*b*) total iron concentration and chlorophyll. Means of July and August, 1948, values.

THE INTERRELATIONSHIPS OF CHLOROPHYLL, SOLUBLE AND TOTAL NUTRIENTS

1. *Soluble iron in relation to chlorophyll and potassium concentrations*

Both reagents (hydrochloric acid saturated with ether and glycerophosphoric acid) gave soluble iron values for the old laminae which increased with the chlorophyll concentration. In general, for the old laminae these soluble iron values correlated better with the chlorophyll concentration than did the total iron values (Fig. 5).

The curve which related both variables was not always linear in the 1949 experiment as the soluble iron concentration continued to increase when the chlorophyll had ceased to do so. The glycerophosphoric acid values showed a similar correlationship to the chlorophyll concentration of the young laminae whereas the hydro-

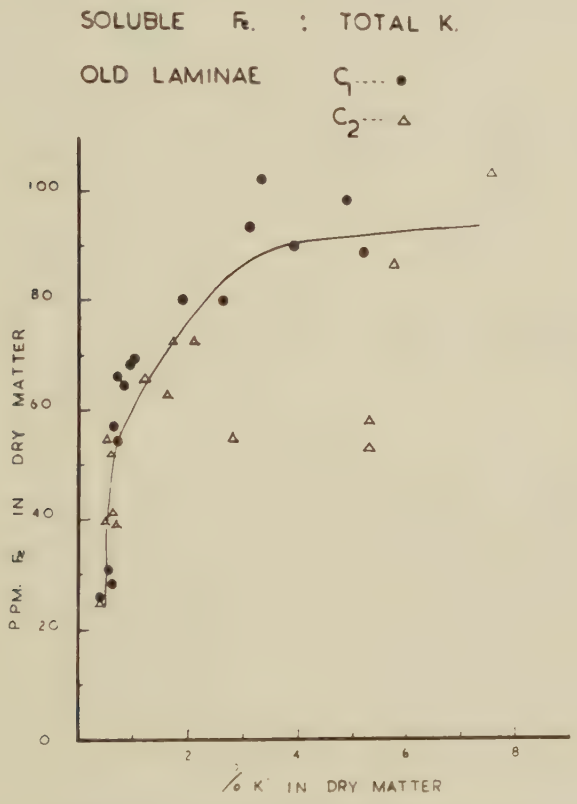

Fig. 6. The variation of iron extracted by 0.1 *N* hydrochloric acid saturated with ether from old laminae, with total potassium concentration. Means of July and August, 1948, values.

chloric acid values did not. Thus the results suggested that both reagents gave a suitable index for the "available" or "active" iron concentration of the old laminae but that the glycerophosphoric acid values only showed promise for the younger laminae.

The iron extracted from the old laminae with hydrochloric acid saturated with ether increased sharply with the total potassium

concentration up to the 1% value and more gradually beyond hist (Fig. 6), the effect was clearer for the C_1 plants. A similar but usually less precise relationship was obtained with the glycerophosphoric acid values for the old laminae.

2. *Young to old lamina ratios for some nutrient elements*

The young/old total potassium ratios decreased sharply at the lower potassium levels with iron application; an overall but less marked decline was also obtained at the higher potassium levels (Fig. 7). Increase in the potassium level consistently decreased the young/old potassium ratio.

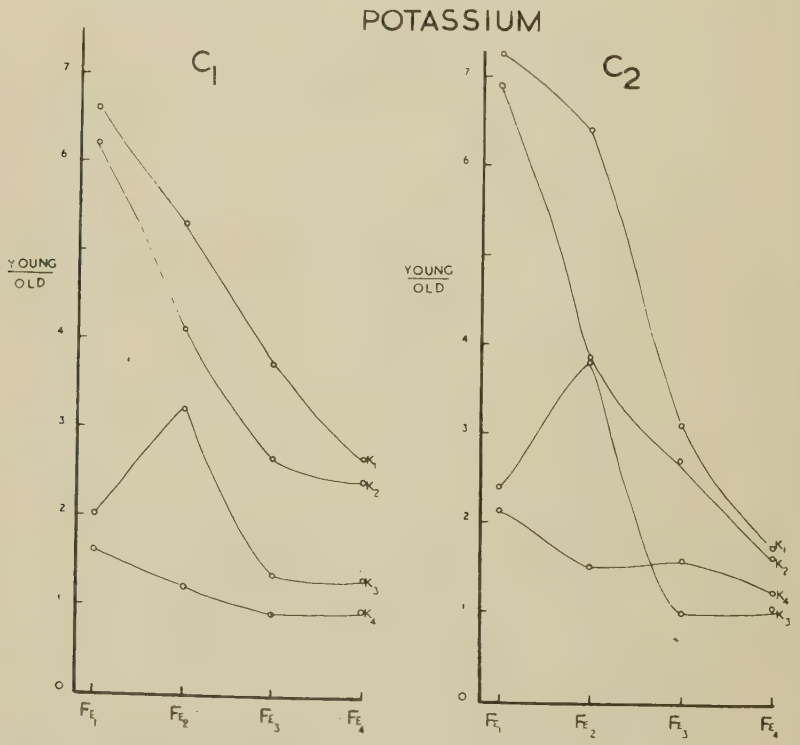

Fig. 7. The variation with iron level of young/old lamina ratios for total potassium concentration. Plotted means of July, August and September, 1948, values. Note sharp decrease in ratio at K_1 and K_2 levels.

3. *Soluble to total ratios for some nutrient elements*

Only a part of the potassium present in the laminae was extracted by Morgan's reagent. The values of soluble/total, potassium

ratios, varied between 0.4 and 1.0. This finding supported observations made by Olsen[16] who concluded that about 30% of the total potassium content in beech leaf cells was adsorptively bound, presumably on the cell proteins. These findings are contrary to the generally accepted view that all the potassium in the cell is readily extractible. The Morgan's-soluble total potassium ratio tended to increase with potassium and to decrease with iron. The physiological significance of this ratio is not clear but it may well be of some importance in chlorotic foliage.

The ratio of Morgan's-soluble to total phosphorus concentration usually decreased on the addition of potassium and increased with phosphate level. The ratio of soluble total, phosphorus, appeared in the 1949 experiment, to decrease as the chlorophyll concentration of the old laminae increased. It was this latter relationship, which, although not well defined, prompted the subsequent investigation of inorganic and organic phosphorus fractions in the leaf as affected by varied levels of iron, potassium and phosphate supply (see "4. *Chemical data* iii.").

4. *The interrelationships of chlorophyll, iron and potassium*

The ratio of total iron to total potassium concentration increased in the young laminae and decreased in the old, with increased chlorophyll concentration. This overall relationship, illustrated in Fig. 8, was obtained by plotting values regardless of treatment levels. If as in Fig. 9, the effects of levels are discriminated it is seen that for the young laminae at the lowest potassium level relatively great increases in the Fe K ratio values were needed to influence the chlorophyll concentration. At high potassium levels the effect of extra iron was diminished and chlorophyll formation was less dependent on the ratio.

The differential response of chlorophyll concentration to varying ratio values according to the age of the laminae reflects the importance of selective sampling in investigations such as this.

5. *The interrelationships of chlorophyll, iron and phosphorus*

The ratio of chlorophyll total Fe usually decreased as the total phosphorus concentration of the young laminae increased (Fig. 10), the old laminae also revealed this effect in the 1949 but not in the 1948 experiment.

The chlorophyll content of young and old laminae increased sharply with increase of the ratio of total iron to phosphorus at the

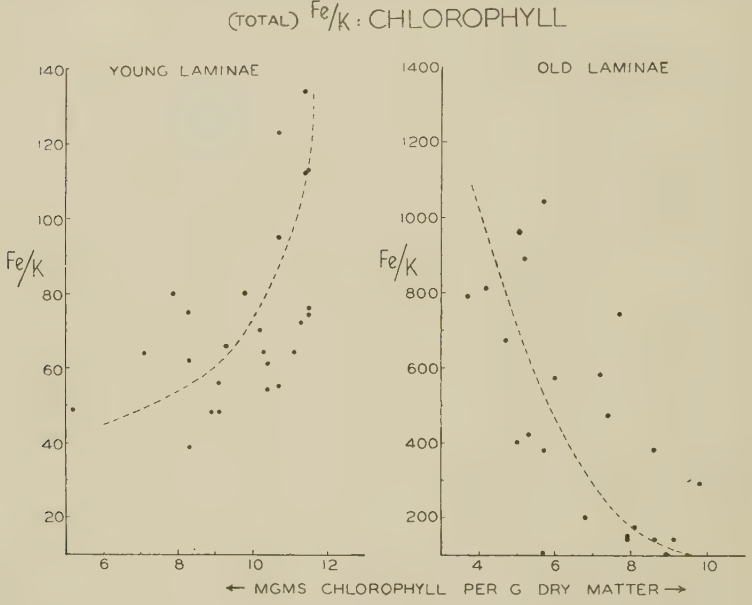

Fig. 8. The variation of the total Fe/total K ratio with the chlorophyll concentration of young and old laminae. July, 1949, sampling.

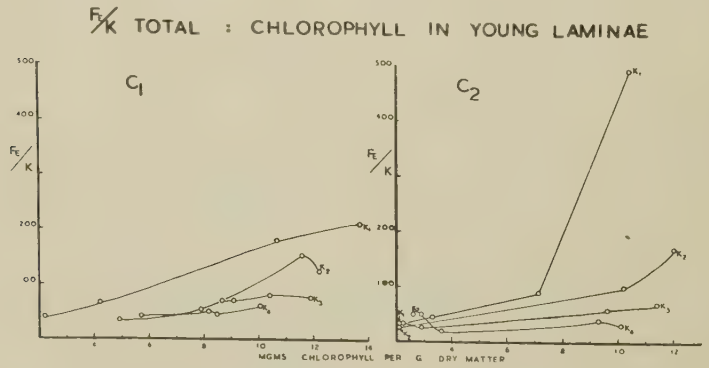

Fig. 9. The variation of the total Fe/total K ratio with chlorophyll concentration of young laminae. Plotted means of July, August and September, 1948, values.

Fe_1 level; the same effect was also obvious at the Fe_1 and Fe_2 levels in the old laminae (Fig. 11). At the Fe_2 and Fe_3 levels in the young

and at the Fe_3 level in the old laminae large increases in the ratio decreased the chlorophyll concentration.

6. *The interrelationships of chlorophyll, potassium and phosphorus*

The relationship between the total K/P ratio and chlorophyll concentration was clearly governed by the iron status and was also probably related to the secondary calcium deficiency effects.

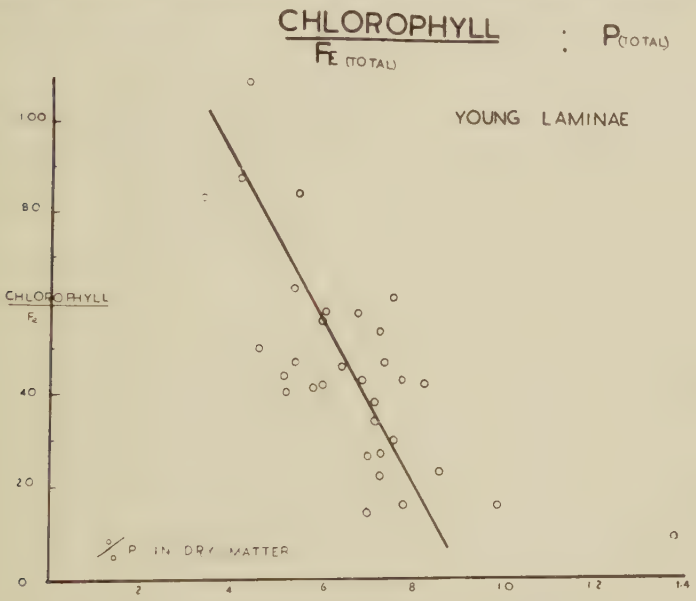

Fig. 10. The variation of the ratio of chlorophyll/total Fe with the total phosphorus concentration of the young laminae. Plotted means of July and August, 1948, values.

Thus the chlorophyll concentration increased as the K/P ratio increased in the young laminae at the Fe_1 level and in the old laminae at the Fe_1 and Fe_2 levels (Fig. 12). A decrease in chlorophyll with increase in the total K/P ratio occurred at the Fe_2 and Fe_3 levels in the young and at the Fe_3 level in the old laminae. The chlorophyll increase with K/P ratio was sharpest at the Fe_1 level in the young but grew steeper with increased Fe level in the old laminae (Fig. 12).

7. *The interrelationships of chlorophyll, calcium and potassium*

The chlorophyll concentration of the young laminae of the K_1 and

K_2 treatments increased as the total Ca/total K ratio increased; this effect was much more striking in the C_2 than in the C_1 treatments (Fig. 13). Slight increases were also obtained in the K_3 treatments but at the K_4 level chlorophyll concentration was not correlated with the Ca/K ratio. These statements describe the effects observed in the 1948 experiment but identical relationships were obtained in subsequent experiments which revealed that the Ca/K ratio only varied with chlorophyll concentration at the deficiency level of potassium, and showed that the relationship was independent of the presence of added calcium carbonate.

In all experiments the chlorophyll concentration of the old laminae showed an overall decrease with increased Ca/K ratio value.

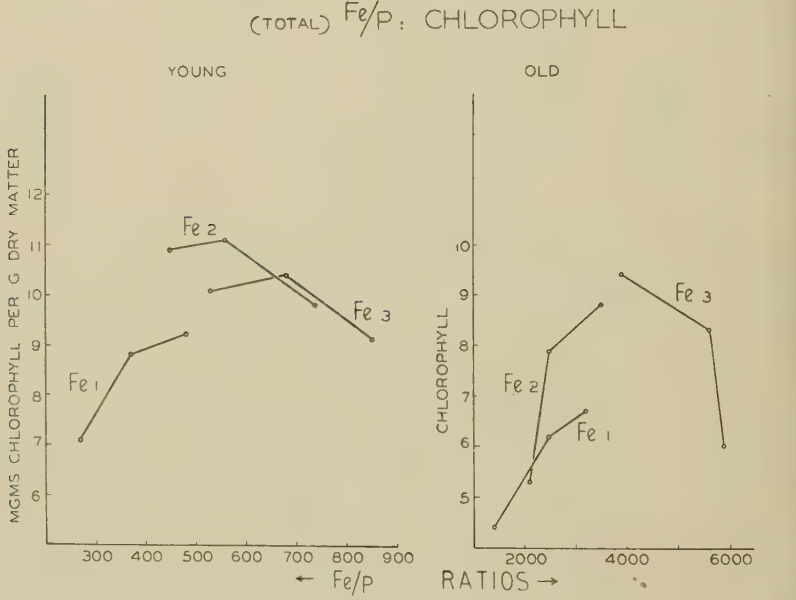

Fig. 11. The relationship between the total Fe/total P ratio and the chlorophyll concentration of young and old laminae. Mean values for July, 1949, sampling. Note similarity between Fe_1 — young and Fe_1 and Fe_2 — old.

DISCUSSION

Many of the visual effects previously described [9]) have now been shown to occur in the potato in the presence of calcium carbonate or high levels of phosphate; in particular the "Fe/K visual response"

(Plate II) and the delaying effect of iron on the inception of potassium deficiency (Plate IV). The response to potassium at deficiency levels of iron could not be attributed to any iron accidentally supplied by the extra potassium salts as analysis of the nutrients did not reveal any such contamination. Neither could the response be attributed to the high sulphate concentration of the higher potassium nutrients; an experiment carried out in 1951, to test this point, showed that at a low level of potassium supply, increased sulphate concentration did not prevent the appearance of chlorosis in the Fe_1P_3 plants. The delaying effect of iron on the inception of potassium deficiency was consistent with the pronounced effect of iron supply on the relative concentrations of potassium found in the young and

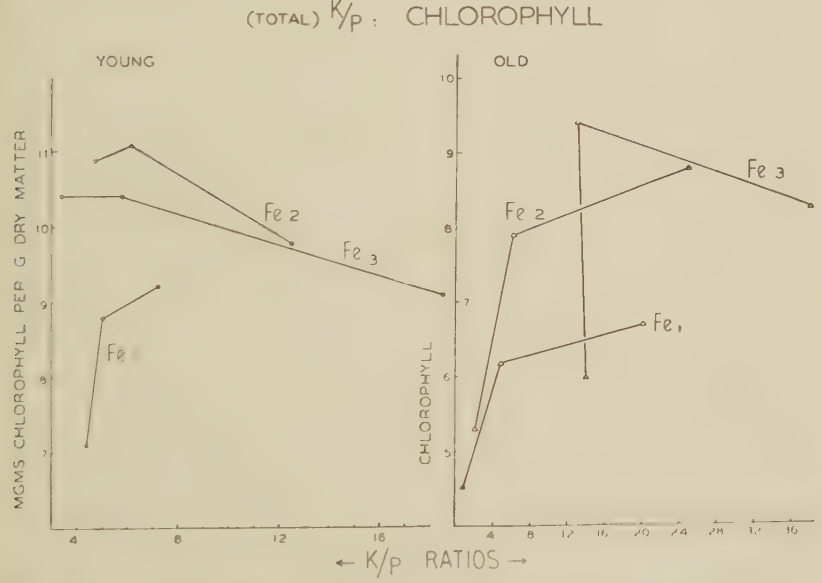

Fig. 12. The variation of the total K/total P ratio with the chlorophyll concentration of young and old laminae. Mean values for July, 1949, sampling.

old laminae (Fig. 7); this reduced young old, potassium ratio, probably reflected the effect of iron on the translocation and distribution of potassium within the plant.

The addition of calcium carbonate increased the pH of the sand and decreased the concentration of iron in the laminae. Calcium

carbonate did not however, cause any fundamental change in the symptoms or relationships observed but merely modified the relative severity, critical concentration or time of appearance.

The Ca/K ratio of the young laminae increased with chlorophyll concentration when the potassium supply was limited but not at the higher potassium levels (Fig. 13). A low Ca/K ratio did not necessarily imply that the foliage was chlorotic as some of the

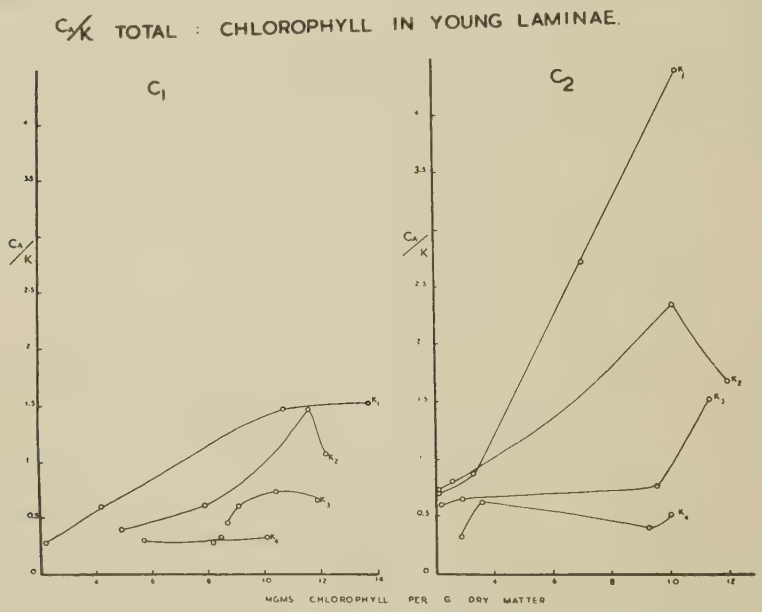

Fig. 13. The variation of the total Ca/total K ratio with chlorophyll concentration of the *young* laminae. Plotted mean of July, August and September, 1948, values.

smallest values obtained were for the higher potassium plants which did not show chlorosis. The relationship between chlorophyll and the Ca/K ratio was emphasised by the addition of calcium carbonate but was also obvious in the C_1 treatments; the relationship was therefore general, as confirmed by the 1949 experiment, and independent of the presence of calcium carbonate. Under field conditions of lime-induced chlorosis where the low Ca/K ratio is generally found, circumstances are also conducive to potassium deficiency and this may be incipient if not visually evident when the Ca/K ratio becomes abnormal. Hence the importance of the Ca/K ratio in

instances of lime-induced chlorosis in the field was now confirmed. The addition of iron in the nutrient raised the Ca K ratio when potassium was deficient and thus illustrated the curative effect of iron on lime-induced chlorosis. It is probable that the interrelationship between the Ca K ratio and chlorophyll concentration of the young laminae reflected the effect of, rather than caused, the different iron status of chlorotic and green plants.

Calcium deficiency symptoms induced by high iron and potassium supplies and associated with a low phosphorus concentration in the laminae (Plate VI) were accompanied by the incidence of medullary necrosis in the tubers (Plate VIII). The medullary necrosis symptoms agreed closely with those described as occurring naturally in the Dutch East Indies by Schwarz [25] and those produced in culture solutions under conditions of calcium deficiency by Van Schreven [33]. Van der Plank [32] found similar necrotic symptoms in tubers grown in strongly acid soils but there were no external or foliage symptoms and control was effected by the application of lime and superphosphate at planting time. It appears that the medullary necrosis reported here was due to a deficiency of calcium accentuated by or associated with a high potassium and low phosphorus status (Table II) and which was accompanied by the characteristic symptoms described for the shoot (Plate VII).

Some of the experiments clearly revealed the parallel and similar effects of iron and potassium in certain aspects of plant nutrition and the opposite effects exerted by phosphate. Thus application of either iron or potassium (Plate II) cured iron deficiency chlorosis, induced and increased the severity of phosphorus deficiency symptoms and accentuated the severity of calcium deficiency symptoms (Plate VII). Extra phosphate supply, however, induced and increased the severity of iron deficiency chlorosis (Plates I and III) and of potassium deficiency symptoms, and reduced the severity of the calcium deficiency. In addition to the above visual effects phosphate exerted a reverse effect to iron and or potassium on dry weight of shoots and roots, incidence of medullary necrosis (Table II), chlorophyll (Table V), phosphorus and calcium (Table XI) soluble and total iron (Tables IX and X) concentrations of the old laminae and total iron concentration of tubers and roots (Tables XII and XIII). Thus, to a large extent iron and potassium exerted similar effects which suggests that both may influence similar physiological

behaviour. It is known that deficiencies of either may cause the accumulation of various soluble organic nitrogen fractions [1] [10] [20] [24] [28] [29] [30] [35] and a depletion of polysaccharides [1] [6] [15] [22] [26] [27]. Both may influence various respiratory systems but there is no known instance in which both iron and potassium perform the same function.

On the other hand there were other effects which emphasized the reciprocal nature of the relationship between iron and potassium. Thus whereas increased potassium supply increased the iron concentration of the laminae, tubers and roots and decreased the severity of iron deficiency symptoms, increased iron supply decreased the potassium concentration of young laminae (Table X), tubers and roots (Table XIII) at the K_1 level and increased the severity of the potassium deficiency symptoms (Plate V).

Increased phosphate supply decreased the concentration of iron and increased iron supply decreased the concentration of phosphorus found in the roots (Table XIII). Despite the decreased iron concentration in the roots, as a result of phosphate addition, the total amount of iron localised in the roots usually increased as a result of the beneficial effect of phosphorus on root production but increased iron supply not only decreased the concentration of phosphorus in the roots but also the total amount localised in them. There was no evidence to suggest that at the high phosphate levels iron accumulated in or was precipitated as ferric phosphate, within the roots.

The role of potassium in mitigating the chlorosis due to lack of iron may be associated with an increased iron (Tables IX and X) and reduced phosphorus concentration (Table XI) of the laminae; these changes were accompanied in the old laminae by an overall increase in the chlorophyll Fe ratio and a decrease in the Fe/K ratio (Fig. 8). Thus it appeared that apart from either the possibility of improved absorption of the existing iron supply or a different distribution of iron within the plant the beneficial effect of potassium on chlorosis may have been due to an enhanced efficiency of iron utilisation for chlorophyll formation.

The favourable effect of potassium level on chlorophyll production in the young laminae at low iron levels (Table V) and the decreasing influence of potassium as the iron supply was increased, also suggested that restricted supplies of iron in the nutrient solution were used more efficiently in the presence of adequate potassium.

The steady decrease with extra potassium of the slope which related the Fe K ratio of the young laminae to the chlorophyll concentration was further evidence (Fig. 9). It implied that at the highest potassium levels only a small increase in the proportion of iron was necessary to give a large increase in chlorophyll *i.e.* the efficiency of the added iron was at its maximum or was no longer limiting. For the young laminae at the Fe_1P_2 and Fe_1P_3 levels large increases in chlorophyll concentration, accompanied by slight increases in iron concentration, were observed on potassium addition thus giving an increased chlorophyll/Fe ratio. When, however, iron was not lacking as at the Fe_1P_1 and the Fe_2 and Fe_3 treatments, potassium addition usually gave a well marked increase in iron concentration and decreased the chlorophyll and phosphorus concentrations of the young laminae consequently the chlorophyll Fe ratio decreased.

The young laminae of the Fe_1P_2 and Fe_1P_3 plants responded to potassium application in the same manner as the old laminae; in both the efficiency of iron utilisation was increased and phosphorus concentration decreased. Thus it might be assumed that this increased efficiency of iron was related to a depression of phosphorus concentration. On this basis the inhibitory influence of potassium on chlorophyll production in the young laminae of the higher iron plants (Table V) may be attributed to the cumulative effect of iron and potassium on the production of calcium deficiency symptoms and a reduced phosphorus status — pathological conditions which hampered chlorophyll production.

Support for the theory that the effect of potassium on increasing the efficiency of iron was due to the reduction in phosphorus was apparent in the increased chlorophyll Fe ratio obtained with decreased phosphorus concentration (Fig. 10) and in the increased chlorophyll concentration of the laminae obtained with increase in the Fe P ratio at deficiency levels of iron (Fig. 11). It was obvious that at the lower iron levels the K P ratio played an important role in chlorophyll formation (Fig. 12). With both the Fe P and K P ratios the young laminae of the Fe_1 plants showed a greater similarity to the old laminae of the Fe_1 and Fe_2 plants than to the Fe_2 and Fe_3 young laminae (Figs. 11, 12).

The 1949 and subsequent experiments showed that when the *overall mean* values for the chlorophyll Fe ratio were plotted against the corresponding phosphorus concentrations a similar relationship to

that depicted for the 1948 experiment (Fig. 18) was obtained. However, scrutiny of the individual results indicated that the chlorophyll/Fe ratio increased with phosphate level when the iron and potassium supplies were *not* limiting growth. Thus an increased phosphorus concentration of the laminae did not *per se* decrease the efficiency of iron utilisation. It appeared that the effect of added potassium in curing chlorosis might be the prevention of iron precipitation by the phosphorus present either by the conversion of the inorganic to organic phosphorus or by some secondary effect on organic acid status and cell sap pH.

If the role of potassium in increasing the efficiency of iron utilisason was partially due to a production of a low tissue pH, the statements of Ingalls and Shive [11], Rogers and Shive [21], Schander [23], Pierre and Bower [18], and Weiss [39] suggest that decrease of the pH would be accompanied by an increase in soluble iron. The results, which have been described, showed, in fact, that the soluble iron increased with potassium supply (Table IX).

Fig. 5, clearly confirms the importance of soluble fractions and illustrates the better relationship obtained between soluble iron and chlorophyll than that obtained with total iron. It was also possible to relate the soluble iron extracted by the hydrochloric acid reagent to the potassium concentration of the old laminae (Fig. 6); this fraction may therefore be regarded as a measure of the efficiency of potassium in enhancing the utilisation of iron in chlorophyll production. The poor correspondence between total iron and chlorophyll may have reflected the analytical difficulties of iron determination in dry material due to the presence of silica [12] or it may imply that the total iron status was not closely related to chlorophyll production. The chlorophyll/soluble or total iron, ratios, decreased markedly with time; this confirmed Bennett's [1] findings. It suggested that the soluble iron (determined with either glycerophosphoric acid or hydrochloric acid reagent) did not accurately represent the fraction of iron active in chlorophyll formation, or that other factors, later in the season, became limiting.

There is evidence in the literature to suggest that increased potassium supply increased organic acid content [5] [22] [34], while increased phosphate decreased it [37]. Chlorosis may also be accompanied by a change in the organic acid constituents of the leaf [10] [13]. Thus it was possible that the effect of potassium on soluble iron concentration and on the utilisation of iron might have operated through its effect on organic acid status which in turn would influence tissue pH.

It was actually found (Table VI) that increased potassium supply usually increased the titratable acidity of the old laminae but decreased that of the young. This beneficial effect on the titratable acidity of the old laminae cannot however be the chief role of potassium in increasing the efficiency of iron utilisation as potassium addition depressed the acidity of the green young laminae in plants which showed the "Fe/K visual response".

The suspected presence of an organic acid which increased in the laminae when plants were supplied with extra potassium and which decreased in iron deficient laminae was of great interest and merits further investigation. The increased occurrence of this acid may conceivably have prevented the immobilisation of iron within the lamina. This interpretation also implies that increased potassium status enhanced the synthesis of this acid within the laminae. The feeding of malic, citric or oxalic acids to iron deficient plants did not cure the chlorosis; in fact, oxalic acid markedly accentuated its severity. The acids, if absorbed, were rapidly converted to other compounds and could not be detected in appreciable amounts in the laminae.

It has been suggested above that one of the roles of potassium in increasing chlorophyll production at iron deficiency levels may be to reduce the ability of phosphorus to immobilise the iron in the lamina. It was therefore interesting to note (see INTERRELATIONSHIPS, 3) that the ratio of Morgan's-soluble total, phosphorus concentration, decreased with increased chlorophyll concentration of the old laminae. It may be inferred that the soluble phosphorus, as determined here, represented the metabolically less active fraction of the total. Further support for this probable role of potassium is given in Table VII. Increased potassium level was found to decrease the concentration of inorganic phosphorus and the ratio of inorganic to organic phosphorus present in the lamina. The preponderance of inorganic phosphorus in iron deficient laminae was therefore probably associated with an inability, due to the lack of potassium, to use the phosphorus in metabolic processes. It may be assumed that a large excess of inorganic phosphorus would effectively immobilise the relatively small amount of iron present in iron deficient laminae. Consequently part of the effect of potassium on iron utilisation appears to be indirect and to be associated with the conversion of inorganic phosphorus to some organic phosphorus-containing

compound which is assumed to be less capable of immobilising the iron in an "inactive" or "unavailable" form.

The foregoing discussion, based mainly on the results of the 1948, 1949 and 1951 experiments, shows that the effect of potassium in ameliorating the severity of iron deficiency chlorosis was partially due to a greater concentration of iron in the lamina and partly due to an increased efficiency of iron utilisation for chlorophyll formation. The 1950 experiment provided information as to whether a greater amount of iron was taken into the plant and the distribution of the various nutrient elements under varied conditions; the remaining part of this discussion deals principally with the results of that experiment.

A the Fe_1P_3 level increased potassium supply decreased the proportion of iron found in the roots but increased it in the tubers and also in the stems and petioles (Fig.2). Increased potassium level increased the "percentage uptake" of iron at the Fe_3P_3 level (Table XV). It is assumed that a similar increased efficiency of iron absorption took place at the Fe_1 level. This was supported by the highly significant increase in "absolute" iron content of the shoot and tubers obtained with increased potassium at all iron levels (Table XIV).

Increased potassium supply also increased the iron content of the complete plant. The effect of potassium on total iron content of the complete plant was not as clearly defined as those obtained for the shoot plus tubers (Table XIV). Inspection of the individual data showed, however, that there was only one exception to the positive effect of potassium on total iron content and that the errors in root analysis, due probably to contamination by the sand, were particularly large. Under these circumstances the conclusion that potassium exerted a real effect would seem to be justified.

It was therefore concluded that one effect of potassium was to increase the mobility of iron within the plant and to facilitate its distribution to the more actively growing regions. A second effect might be inferred — namely that potassium increased the efficiency of iron absorption by the plant.

The addition of phosphate at the Fe_1K_1 level decreased the proportion of iron found in the tubers, stems and petioles and in the bottom laminae but increased it in the roots and in the upper laminae (Fig. 2). Increased phosphate supply also increased the

"percentage uptake" of iron at the Fe_3K_3 level but decreased it at the Fe_3K_1 level (Table XV). If the same effect applied at the Fe_1 level, it would accord with visual observation and would explain the occurrence of chlorosis on the addition of phosphate at the Fe_1K_1 level and the production of tall vigorous plants at the Fe_1K_3 level.

Hence it was concluded that the effects of phosphate addition at the Fe_1K_1 level were to retain the iron in the roots, owing to increased root production, and possibly to "immobilise" it in the upper laminae, where chlorosis was obvious. It also interfered with iron absorption at low potassium levels.

Increased iron supply decreased slightly the potassium content of the complete plant but this effect was not significant (Table XIV). Added iron, however, markedly influenced the distribution of potassium within the plant. It increased the proportion found in the tubers but decreased it in the laminae and stems and petioles (Fig. 3). This effect became more marked with time, especially at the K_1P_2 level. This altered distribution was consistent with both the greater ultimate severity of the potassium deficiency and larger tuber production at the Fe_3 level.

Increased iron or potassium supply decreased the proportion of phosphorus found in the roots and laminae but increased it in the tubers (Fig. 4). This distribution was apparently conducive to the appearance of phosphorus deficiency symptoms.

The experimental evidence suggested that increased potassium status may cause the migration of iron and phosphorus from the plant to the mother tuber during the growth period (Table XV). The application of phosphate, however, usually caused an increase in the absolute amount of iron withdrawn from the seed tuber. These effects were paralleled by the influence of treatments on the relative distribution of nutrients to the newly formed tubers. Thus the proportion of iron and phosphorus generally increased with iron or potassium application but decreased with phosphate (Figs. 2, 4). The addition of iron increased the proportion of potassium found in the tubers but potassium or phosphate generally decreased it (Fig.3).

It was remarkable that, despite the free drainage of each pot, the efficiency of potassium absorption, as expressed by the "percentage uptake", was 80% or more, in the K_1 treatments. In the $Fe_1K_1P_1$ treatments the absorption was 100% - allowing for experimental

error (Table XV). The efficiency of the plants in absorbing phosphorus was much less.

The addition of iron decreased the efficiency of potassium absorption at the P_1 level but increased it at the K_1P_3 level and had no effect at the K_3P_3 level (Table XV). The addition of potassium decreased at the Fe_3P_1 but increased at the Fe_3P_3 level the "percentage uptake" of iron. Hence the effect of iron on the efficiency of potassium absorption and of potassium on the efficiency of iron absorption depended on the phosphate level. The relationship between iron and potassium could not therefore be defined as a simple antagonism or synergism. In a similar manner the effect of phosphate on the "percentage uptake" of iron depended upon the K level.

On the other hand the interrelationship between potassium and phosphorus approximated to that of synergism. Increase of potassium level increased the percentage uptake of phosphorus; phosphate addition increased the uptake of potassium at the K_3 level, but reduced it at the Fe_1K_1 level (Table XV). In spite of the increased efficiency of phosphorus uptake at the Fe_1P_1 level due to potassium, the visual response was one of increased severity of phosphorus deficiency owing to the more widely disturbed nutrient balance in the plant.

The results showed clearly that a discussion of the effect of treatment on the nutrient status of the plant depended greatly upon both the region sampled and the age of the plant. The interaction between iron and potassium and between iron and phosphorus were especially subject to this qualification. In addition there was a modifying effect of the third element on the interaction of the other two.

GENERAL SUMMARY AND CONCLUSIONS

1. Iron and potassium were shown to be interrelated in the metabolism of the potato plant. The nature of this interrelationship was dependent on the level of phosphate supply.

2. When iron supplies were limiting, chlorosis was induced or accentuated by the addition of calcium carbonate, by the addition of phosphate, or by a reduction in the supply of potassium.

3. Iron deficiency chlorosis was cured by the addition of high levels of potassium. This response to potassium was associated with an increased iron concentration in the lamina and with a greater efficiency of iron utilisation in chlorophyll formation.

4. It was suggested that the effect of potassium on the iron content of the lamina was due to an increased mobility of iron in the plant, inferred from a study of nutrient uptake and distribution. This enhanced mobility was associated with a reduction in the proportion of iron localised in the roots in the presence of a plentiful supply of phosphate.

5. The increased efficiency of iron utilisation was attributed both to the depression in the total phosphorus concentration and also to the decrease in the concentration of inorganic phosphorus and in the ratio inorganic/organic phosphorus found in the lamina.

6. The chlorophyll concentration of the young laminae of potassium deficient plants varied with the Ca K ratio; this relationship was independent of the presence of calcium carbonate and was dependent on the iron status of the plant.

7. Iron status governed the distribution of potassium within the plant; high iron levels favoured its translocation to the tubers at the expense of the young shoot regions and altered the gradient of concentration between young and old laminae.

8. The work as whole illustrated the importance of the age of the tissue and of the selection of the morphological region sampled in assessing nutrient interrelationships, and of the modifying effect of any one nutrient on the precise nature of the interaction between the others studied.

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REFERENCES

1) Bennett, J. P., Iron in leaves. Soil Sci. **60**, 91 (1945).
2) Comar, C. L., Analysis of plant extracts for chlorophyll *a* and *b* using a commercial spectrophotometer. Ind. Eng. Chem. Anal. Ed. **14**, 877 (1942).
3) Comar, C. L. and Zscheile, F. P., Analysis o fplant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method. Plant Physiol. **17**, 198 (1942).
4) Comar, C. L., Benne, E. J. and Buteyn, E. K., Calibration of a photoelectric colorimeter for the determination of chlorophyll. Ind. Eng. Chem. Anal. Ed. **15**, 524 (1943).

5) Cooil, B. J., Potassium deficiency and excess in Guayule. 2. Cation-anion balance in the leaves. Plant Physiol. **23**, 403 (1948).
6) Cooil, B. J. and Slattery, M. C., Effects of potassium deficiency and excess upon certain carbohydrate and nitrogenous fractions in Guayule. Plant Physiol. **23**, 425 (1948).
7) Hewitt, E. J., A technique for large scale pot sand cultures. Ann. Rept. Long Ashton Research Sta. 1946, 37 (1947).
8) Hewitt, E. J., The resolution of the factors in soil acidity: some effects of manganese toxicity. Ann. Rept. Long Ashton Research Sta. 1946, 50 (1947).
9) Hewitt, E. J., and Bolle-Jones, E. W., Studies in iron deficiency of crops. 2. Preliminary experiments on the interrelationships of iron and potassium in the potato plant. J. Hort. Sci. **28**, 185 (1953).
10) Iljin, W. S., Der Stoffwechsel bei der Weinrebe während der Kalkchlorose. Die Gartenbauwiss. **17**, 338 (1943).
11) Ingalls, R. A. and Shive, J. W., Relation of hydrogen ion concentration of tissue fluids to the distribution of iron in plants. Plant Physiol. **6**, 103 (1931).
12) Mason, A. C., The estimation of iron in plant material. Ann. Rept. East Malling Research Sta. 1950, 124 (1951).
13) McGeorge, W. T., Lime induced chlorosis. Relation between active iron and citric and oxalic acid. Soil Sci. **68**, 381 (1949).
14) Nicholas, D. J. D., The application of rapid chemical tests to the diagnosis of mineral deficiencies in horticultural crops. Parts I and II. J. Hort. Sci. **24**, 72 (1948).
15) Nightingale, G. T., Physiological chemical functions of potassium in crop growth. Soil Sci. **55**, 73 (1943).
16) Olsen, C., Adsorptively bound potassium in Beech leaf cells. Physiol. Plantarum **1**, 136 (1948).
17) Oserkowsky, J., The quantitative relation between chlorophyll and iron in green and chlorotic pear leaves. Plant Physiol. **8**, 449 (1933).
18) Pierre, W. H. and Bower, C. A., Potassium absorption by plants as affected by cationic relationships. Soil Sci. **55**, 23 (1943).
19) Quanjer, H. M., Waarnemingen over kringerigheid of „vuur" en over „net necrose" van aardappelen. Tijdschr. Plantenziekten **32**, 97 (1926).
20) Richards, F. J. and Templeman, W. G., Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Botany **50**, 367 (1936).
21) Rogers, C. H. and Shive, J. W., Factors affecting the distribution of iron in plants. Plant Physiol. **7**, 227 (1932).
22) Roy, W. R., Effect of potassium deficiency and of potassium derived from different sources on the composition of the juice of valencia oranges. J. Agr. Research **70**, 143 (1945).
23) Schander, H., Gedanken über Unterschiede und Übereinstimmungen der Chlorosiserscheinungen von Lupinen und Holzgewachsen. Die Gartenbauwiss. **17**, 304 (1942).
24) Schmalfuss, K., Untersuchungen über den Eiweissstoffwechsel von Kalimangelpflanzen. Phytopathol. Z. **5**, 207 (1932).
25) Schwarz, B., De roestvlekkenziekte van aardappelknollen in Nederlandsch Oost Indië. Tijdschr. Plantenziekten **32**, 321 (1926).
26) Sideris, and Young, H. Y., Effects of iron on chlorophyllous pigments, ascorbic acid, acidity and carbohydrates of *Ananas Comosus* supplied with nitrate or ammonium salts. Plant Physiol. **19**, 52 (1944).
27) Sideris, C. P. and Young, H. Y., Effects of potassium on chlorophyll, acidity, ascorbic acid and carbohydrates of *Ananas Comosus*. Plant Physiol. **20**, 649 (1945).

Sideris, C. P. and Young, H. Y., Effect of iron on certain nitrogenous fractions of *Ananas Comosus* (L). Merr. Plant Physiol. **21**, 75 (1946).

Sideris, C. P. and Young, H. Y., Effect of potassium on the nitrogenous constituents of *Ananas Cosomus* (L) Merr. Plant Physiol. **21**, 228 (1946).

Steinberg, R. A., Bowling, J. D. and McMurtrey, J. E., Accumulation of free amino acids as a chemical basis for morphological symptoms in tobacco manifesting frenching and mineral deficiency symptoms. Plant Physiol. **25**, 279 (1950).

Thorne, D. W. and Wallace, A., Some factors affecting chlorosis on high lime soils. 1. Ferrous and Ferric Iron. Soil Sci. **57**, 299 (1944).

Van der Plank, J. C., "The potato in health and disease". (1936), pages 235 and 326 by Whitehead, McIntosh and Findlay, (1945).

Van Schreven, D. A., Kalkgebrek als oorzaak van mergnecrose bij aardappelknollen. Tijdschr. Plantenziekten, **40**, 226 (1934).

Vladimirov, A. V., Effect of potassium and magnesium sulphates and chlorides upon the formation of oxidised and reduced organic compounds in plants. Soil Sci. **60**, 377 (1945).

Wall, M. E., Nitrogen and carbohydrates metabolism in potassium deficient plants supplies with either nitrate or ammonium nitrogen. Soil. Sci. **49**, 393 (1940).

Wallace, T., "The diagnosis of mineral deficiencies in plants". (H. M. Stationery Office) (1943).

Ward, D. E., and Petrie, A. H. K., The drifts in organic acid content of tobacco leaves in relation to phosphorus supply and topping. Australian J. Exp. Biol. Med. Sci. **18**, 21 (1940).

Weil-Malherbe, H. and Green, R. H., The catalytic effect of molybdate on the hydrolysis of organic phosphate bonds. Biochem. J. **49**, 286 (1951).

Weiss, M. G., Inheritance and physiology of efficiency in iron utilisation in soybeans. Genetics **28**, 253 (1943).

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A PRELIMINARY STUDY OF ALUMINIUM AND THE TEA BUSH

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All bio-geochemical *) studies should include a detailed account of the uptake and physiological effects of the element concerned on at least one plant species. The aluminium relationships of the garden hydrangea, *H. macrophylla*, were explored in this respect to a limited extent by the writer [3]) twenty years ago but as a corollary to a comprehensive survey of aluminium in the plant world [4]) [6]) a more detailed investigation seemed desirable. Since the tea bush, *Camellia sinensis*, is the aluminium-plant with the greatest economic value it was chosen as the species to study. It was believed that its connexions with aluminium might eventually have some bearing on its cultivation. The work described below was largely undertaken at Rothamsted Experimental Station in 1948–50 as part of a Colonial Development and Welfare Research Scheme and was later followed up in Uganda.

HISTORICAL

Bertrand and Levy [2]) in 1931 appear to have been the first to record a high aluminium content for tea. They examined 76 species of food and garden plants and found that prepared Ceylon tea contained 465 ppm †) aluminium as against a mean of 11 ppm for the rest. The next year Yoshii and Jimbo [38])

*) Bio-geochemistry may be simply defined as the natural history of individual chemical elements [17]).

†) All analytical data cited in this paper have been recalculated to parts per million (ppm) or milligrams per kilo of oven dried material.

published the results of their survey of the occurrence of aluminium in the plant kingdom. This included old leaves of the tea bush which contained 2370 ppm aluminium. It must be stated here that the tea family (*Theaceae*) had been shown to be aluminium accumulating much earlier in 1922 by Hallier[15]) and in 1927 by von Faber[7]) but they did not test the tea bush. In 1934 the present writer determined aluminium in old leaves from the tea bush growing in one of the economic greenhouses at the Royal Botanic Gardens, Kew. Only 392 ppm aluminium was found. High figures are cited in American literature by Robinson[28]), as much as 9300 ppm aluminium being found by Hou. McMurtrey and Robinson[22]) in a review of neglected soil constituents state that concentrations nearly as high as 20,000 ppm are possible although they give no specific examples. Russian workers have only recently been aware of the ability of the tea bush to take up large amounts of aluminium. The earliest mention of this is by Polynov[26]) in 1944 and although he refers to it later[27]) he does not quote any figures. Quantitative data for aluminium in Russian tea, as far as can be ascertained, have only very recently been published. Parfenova and Troitskii[24]) in 1951 recorded 3220 ppm aluminium for healthy mature tea leaves and 1310 ppm for unhealthy leaves.

CONSTANCY OF ALUMINIUM IN THE TEA BUSH

In order to verify the foregoing and to obtain some idea of the constancy of high aluminium uptake by the tea bush a series of samples were taken from Kew Herbarium specimens which had originally grown in countries not included above. The results *) are tabulated below.

It is evident from Table I and the results of previous workers that considerable variation occurs in the amount of aluminium accumulated by the tea bush but it is a constant feature. This variation may be due to differences in age of leaf, age of tree, genetic constitution, rainfall, altitude or soil. The effect of these factors will now be assessed.

*) Details of the method of analysis are given in the appendix.

TABLE I

Kew Herbarium specimens		
Location	Collector	Aluminium ppm
India		
Kodaikanal, Pulney Hills, South India	Souliere	8,360
Pashok, Darjeeling, North India	—	*ca* 10,000
China		
Yunnansen	Maire 1094	7,860
Mid-west Yunnan	Forrest 26029	6,820
North Yunnan	Forrest 27384	8,850
No locality	Fortune 165	2,650
Tibet		
South East Tibet	Forrest 18886	11,500

ALUMINIUM CONTENT AND AGE OF LEAF AND TREE

The low figure of the commodity sample analysed by Bertrand and Levy indicates that young leaves do not contain anything like the amount of aluminium present in old leaves. In order to confirm this and at the same time study the other factors concerned with aluminium uptake three extensive series of samples were obtained from Ceylon, Tanganyika and Kenya respectively which included as many different jats and clones as possible. A selection of the results obtained is presented for each series in Tables II, III and IV.

It is abundantly clear from the above data that the tea bush takes up aluminium throughout its life, even when left to grow into a tree and stores it in the leaves. The greater part of the storage takes place during the seven months following maturity (Table II). Young leaves contain only about 100 ppm and this increases with genetic variation to between 5,000 and 16,000 ppm in leaves about to fall. In the flush (*i.e.* the commercial part of the plant) the aluminium content increases progressively from bud to mature leaf — from 50 ppm to 1500 ppm. A normal well plucked flush (bud and two leaves) would contain between 150 and 250 ppm which is perfectly harmless to consumers of the beverage. Aluminium content might well be used as an index of "Goodness of Pluck" for the rise from the second leaf to the third and fourth is steep:

TABLE II

Ceylon: St Coombs, Talawakelle								
Clone	Mean yield lbs/ acre	Blister blight	Age After pruning months	Aluminium, parts per million				
				Flush	Banji 1st leaf	Banji 2nd leaf	Mature	
9	1033	0–1	7	170	44	132	10500	
			25	39 } 116	83 } 84	213 } 229	8650 } 9750	
			37	138	125	343	10100	
25	1753	3–4	7	lost	300	840	8500	
			25	130 } 125	133 } 221	582 } 711	9300 } 11300	
			37	120	260	spoilt	16000	
26	523	2–3	7	140	lost	335	10600	young
			25	107 } 118	180 } 180	380 } 403	1205 }	leaves
			37	100	180	495	1285	?
777	991	1–2	7	74	197	334	5150	
			25	93 } 100	201 } 182	352 } 368	14300 } 9460	
			37	133	149	420	8940	
934	1029	1–2	7	102	118	272	7400	
			25	282 } 209	285 } 212	625 } 534	9650 } 7830	
			37	243	232	705	6430	
1114	1170	1–2	7	292	139 } 166	386 } 306	5560	
			25	121 } 207	194 }	225 }	7900 } 7620	
			37	208	187		9400	
1294	676	4	7	43	105		2780	
			25	57 } 66	59 } 101		1580 } 3480	
			37	99	140		6100	
1526	—	-	7	46	492		7550	
			25	35 } 82	565 } 526		7200 } 7370	
			37	165	89		7350	

Observations:

		Mature	Banji	Bud
Clonal Features:	High Al	Clones 25, 9, 777, 26	25, 934, 1526	934
	Medium Al	,, 931, 1114, 1526		
	Low Al	,, 1294		

Notes: Yields are for the 1st pruning cycle.

Blister blight 0 = Disease absent.
1 = White blisters on leaves.
2 = Many white blisters on leaves, no stem infection.
3 = Many young stems damaged, but not killed throughout length.
4 = Young stems seriously damaged, many completely destroyed.

Rainfall: 95 inches.
Soil: Yellowish brown on Red Loam.
Altitude: 4,500 ft.

TABLE III

Tanganyika

Variety or jat	Location	Age years	Aluminium ppm		Phosphorus ppm	
			Youngest	Oldest	Youngest	Oldest
Assamica	Amani Village	47	4640	7550 (9)	1120	1200
Unknown	Amani Herbarium	46	7500	8400 (9)	1050	800
Unknown	Amani, Tea Corner	36	2520	4500 (9)	1430	1100
Manipuri ex Nyasaland	Kwamkoro	20	5200	5480 (9)	1370	1420
Bazaloni ex Pangledjar Java	,,	20	4380	6200 (7)	1440	1800
Jatinga–Manipuri (thick)	,,	20	6750	8600 (7)	2430	1280
Manipuri ex Ceylon	,,	20	5160	15400 (6)	1020	1020
Dahootea Assam	,,	20	8130	10800 (7)	945	1290
Dahootea Assam	Amani	17	2570	8350 (7)	985	930
Jatinga Assam	,,	17	9420	17100 (7)	880	960
Jingamira Assam	,,	16	7950	12600 (7)	1500	1570
Manipuri ex Tingamira Est.	,,	16	10800	11700 (7)	2000	1750
Assamica ex Keroyalla Est.	,,	16	2880	10400 (6)	1260	1670
Black Manipuri	Monga, Karimi Est.	4	61	591 (4)	2500	1310
Rajghur	,, ,, ,,	4–5	577	2880 (4)	2680	875
Pure Assamica	,, ,, ,,	4–5	711	1950 (4)	2980	1500
China jat	,, ,, ,,	1	214	8540 (4)	4620	1560
Assamica	Amani Embryo	47	13			
,,	,, Testa	47	69			
,,	,, Carpel	47	293			

Notes: Samples were taken from long mature shoots; figures in brackets are the number of leaves per shoot, all of which were analysed, the gradation in Al and P were usually quite regular from youngest to oldest leaf.

Rainfall: 76 inches. Soil: Red Loam. Altitude: 2989 feet.

TABLE IV

Kenya

ety or jat	Site	Alt., feet	Rain, inches	Age, years	Soil		Al ppm	P ppm	Mn ppm
				Large mature leaves					
	Kaisugu Est.	7300	68	12	Black on murram		3100	1670	5600
	,, ,,	7300	68	13	,, ,, ,,		2950	1670	4000
	,, ,,	7300	68	22	,, ,, ,,		5120	1810	1800
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		1900	1100	5500
	,, ,,	6800	65	20	,, ,,		10200	2300	8800
	Kapkimolwa Est.	6200	57	4			350	1390	170
	Kericho Est.	6950	71	50	Red loam		3310	1710	5900
	Buret T. Co.	6200	65	25	Red on murram		2060	1800	3000
				Small half-mature leaves					
	Ngambuya	5500	40	7	Red loam		3200	1460	n.d.
	Kaganjo	5500	40	14	,, ,,		3320	1480	1270
	Kanyenyeni	6600	60	2	Volcanic tuff		2050	1950	n.d.
	Karuri	7100	65	18	,, ,,	young leaf	2240	2140	n.d.
	Igonta, Fort Hall	6200	50	14	Red loam	young leaf	1930	2580	n.d.
	Maguru School	5700	45	9	,, ,,		1100	2000	n.d.
	Gatara	6300	52	10	,, ,,		625	2690	n.d.
				Young leaves					
	Kagunduini	6500	48	20	Red loam	bud, 1st leaf, 2nd leaf	455	2760	1340
	,,	6500	48	20	,, ,,	3rd leaf	1040	2440	2300
,,	Karuri Plot	7100	65	18	Volcanic tuff	thin banji leaf	312	2670	1360
	Manunga	7200	65	2	,, ,,	tip	712	3540	620
,,	,,	7200	65	2	,, ,,	3rd leaf	1250	3470	n.d.
	Gathagara	6300	50	25	,, ,,	bud, 1st leaf, 2nd leaf	712	4170	715
,,	,,	6300	50	25	,, ,,	3rd leaf	1250	3050	770
,,	,,	6300	50	20	,, ,,	bud, 1st leaf, 2nd leaf	680	3160	1180
,,	,,	6300	50	20	,, ,,	3rd leaf	895	1640	1850
Indigenous	Limuru	7340	52	19	Chocolate loam	bud, 1st leaf	115	2220	0
,,	,,	7340	52	19	,, ,,	2nd leaf	440	2150	0
,,	,,	7340	52	19	,, ,,	bud, 1st leaf	295	3070	0
,,	,,	7340	52	19	,, ,,	2nd leaf	580	2160	0
						bud, 1st leaf	250	4050	0
						2nd leaf	520	—	

not determined.

250 ppm to 900–1500 ppm. Contamination with dust is inevitable but it can easily be removed by vigorous and repeated shaking with 50% alcohol or acetone acidified to about 1% with nitric acid; rubbing whole leaves with cotton wool soaked in this solution followed by vigorous shaking completely removes both dust and soil splash.

ALUMINIUM AND GENETIC CONSTITUTION

The results for Ceylon and Tanganyika show that certain clones and strains or jats are more vigorous accumulators of aluminium than others. For example clones 25 and 777 from Ceylon and the Jatinga Assam jat from Tanganyika approach the maximum figure of 20,000 ppm quoted by McMurtrey and Robinson. The Indian Tea Research Institute at Tocklai, Assam recognises three distinct sub-species of tea: — *Camellia sinensis* var. *assamica*, the large leafed Indian tea; var. *sinensis*, the small leafed China tea and var. *cambodiensis*, the thick leafed, drooping tree not used in commerce. It was considered that samples from these extreme varieties grown on the same kind of soil might show extreme differences in aluminium content. Material was obtained from

TABLE V

Assam, India
Tocklai Experimental Station, Cinnamara

Variety	Stem	Young leaves	Banji leaves	Mature leaves	Dropping leaves
			Aluminium ppm		
cambodiensis	130	309	396	4500	19500
sinensis	188	155	166	4000	10000
assamica	112	331	512	2820	4450
			Phosphorus ppm		
cambodiensis	445	3340	2700	1240	1610
sinensis	425	3180	3190	1090	565
assamica	438	3930	3010	1800	1730
			Manganese ppm		
cambodiensis	190	760	800	2000	1650
sinensis	410	480	770	3800	2100
assamica	140	560	700	1500	1200

Altitude: 284 feet.
Rainfall: 81 inches.
Soil: Yellowish brown alluvial loamy sand.

Tocklai and analysed for aluminium. The results are submitted in Table V. The genecontrolled nature of aluminium uptake is again strikingly confirmed.

ALUMINIUM AND BLISTER BLIGHT

Another character of the tea bush which is controlled by genes is its susceptibility to the disease "blister blight". It was suggested by Eden [10]) and Lamb [19]) that aluminium might be connected with this disease, but in the first set of Ceylon samples (Table II) there was no correlation. A later set of samples was secured from the Tea Research Institute of Ceylon to examine aluminium content and extremes of resistance and susceptibility to blister blight. The results obtained showed clearly that the differences in aluminium content are not related to blister blight in any direct manner. The results are tabulated below: —

TABLE VI

Ceylon, St. Coombs, Talawakelle	
Blister blight	Aluminium ppm *)
Highly resistant	2100
Highly susceptible	5500
Susceptible but non-fermenter	1230

*) Mean of three determinations each.

ALUMINIUM AND TEA YELLOWS

It is appropriate here to consider aluminium accumulation of the tea bush and the sulphur deficiency symptoms known as "tea yellows" [34]). Since excess of aluminium in many other genera produces a permanent yellowish green colour in the dry leaves [5]) it was thought that some, at least, of the yellowness in tea yellows might be linked with aluminium. A set of samples was accordingly obtained from the Mlange Tea Experimental Station, Nyasaland and analysed in the usual manner. The most noteworthy feature of the results is a very definite increase of aluminium in the affected flushes — a mean of 640 ppm as against 380 ppm in the healthy flushes. The manganese contents of the old leaves of the healthy plant are the highest ever recorded for any healthy species. The high phosphorus figures for the slightly

and moderately affected leaves are probably due to fertilizer treatments.

TABLE VII

Nyasaland Mlange Tea Experimental Station				
Yellows status	Age of leaf	Al ppm	Mn ppm	P ppm
Healthy	Flush, first leaf	260	1100	2810
	Flush, second leaf	500	630	2870
	Mature	7900	9150	1500
	At point of dropping	10600	9700	1450
Slightly affected	Flush, both leaves	390	1400	2900
	Mature	4900	3000	4100
	Older	12500	3700	6400
Moderately affected	Flush, first leaf	360	1100	2550
	Flush, second leaf	2800	1700	2740
	Mature	7300	3570	7300
	Older	5400	3000	3300
Severely affected	Flush, both leaves	1300	400	3800
	Flush, both leaves	1550	1100	2070
	Mature	1670	1600	1900
	At point of dropping	7300	3500	1100

Altitude: 2125 feet.
Rainfall: 62 inches.
Soil: Red Loam. pH 5.2–6.0.

DISTRIBUTION OF ALUMINIUM IN THE TEA BUSH

Before discussing the relationships between aluminium uptake and climate or soil it is relevant first to study some of the more simple physiological phenomena of distribution within the bush and interactions with other elements. The data in Table V indicate that aluminium is likely to be stored largely in the leaves just as it is in the hydrangea and other plants [2] [3]. In order to confirm this, tea seedlings were grown to about 8 inches tall at Kew and then fed with strong solutions of aluminium and manganous sulphates, (2,000 ppm Al and Mn, 8 doses; 10,000 ppm Al and Mn, 2 doses). After four months the plants were pulled up and sectioned for analysis. Manganese and phosphate were determined in each portion of the plant at the same time as the aluminium. Unfortunately only one replication of four treatments was possible owing to shortage of seedlings. Whatever differences that did occur were so striking that it was considered worthwhile to analyse completely and separately one plant from each treatment. Apart

from the distribution data some interesting points were revealed concerning aluminium in relation to manganese and phosphate. Detailed results are given in Table VIII and a summary of the whole experiment in Table IX.

A glance at Table VIII at once reveals that the leaves are the organs where aluminium is stored, very little occurs in the stems or woody roots. The secondary roots contain more aluminium but some of this is soil contamination which could not be washed off. The same distribution pattern occurred in all four plants and also in those examined by Parfenova and Troitskii[24]). Tea fruits from Tanganyika contained only traces of aluminium (reported in Table III).

ALUMINIUM AND ITS RELATIONSHIPS WITH PHOSPHORUS AND MANGANESE

The phosphorus determinations made on the Tanganyika, Kenya and Kew pot samples all indicate that the distribution and mobility of phosphorus in the tea bush is quite normal despite the presence of a large excess of aluminium and manganese, although both elements might conceivably immobilise phosphorus as insoluble phosphates. Growing points such as shoot and root tips contain the largest amounts of phosphorus and this diminishes with age of leaf by translocation through the stem. A certain amount of storage takes place in the stem. The fact that both aluminium and manganese contents of the youngest leaves, stems and woody roots are extremely low probably accounts for the non-interference of these elements with phosphate movement.

With regard to manganese it is interesting to note that the plant treated with manganous sulphate was the tallest and had the largest and thinnest leaves which were slightly chlorotic at the end of the experiment. Unlike the others this plant never entered the dormant "banji" state during the short duration of the experiment but grew continuously.

Certain trends are suggested by the data summarized in Table IX. The aluminium treated plant which was the healthiest and greenest of the set, had the highest weight of tops and underground parts, and the manganous sulphate treated plant although it showed signs of chlorosis was better in both respects than the controls. The

TABL

Kew p

	Control				Aluminium treated			
	Al ppm	Mn ppm	P ppm	Wt g	Al ppm	Mn ppm	P ppm	Wt g
Leaf 1	345	575	1970	.196	920	840	1400	.293
,, 1 + 2								
,, 2 + 3	478	850	1770	.625	1500	1300	1140	.750
,, 3								
,, 3 + 4								
,, 4								
,, 4 + 5	564	810	2260	.493	2960	2610	1760	.710
,, 5								
,, 5 + 6								
,, 6 + 7	596	1170	2290	.475	1630	1650	895	.846
,, 8								
,, 8 + 9	940	1840	2130	.488	1780	1860	1120	.843
,, 9								
,, 10	1620	2010	2030	.026	2100	2160	880	.335
,, 10 + 11								
,, 11					2440	1300	1030	.077
,, 11 + 12	1590	3000	1300	.257				
,, 12 + 13					2650	3000	750	.531
,, 13	2320	2570	1080	.12				
,, 14 + 15	2910	2100	1360	.017	3890	4650	510	.211
,, 16					4660	2280	545	.100
,, 16 + 17								
,, 17 + 18					7500	2750	712	.010
,, 18 + 19								
,, 20								
Flower buds					230	180	2290	.044
Green stems	61	100	2830	.025	296	18	2760	.041
Brown stems	26	85	2120	.867	198	15	2180	1.325
Woody stems	72	108	1600	.814	285	110	855	.884
Stem bases	115	83	1270	.248	208	107	1010	.342
Stems below	76	<16	1470	.143	306	68	1610	.181
Root stocks	174	28	1550	.659	96	41	1550	2.081
Primary roots	280	55	2050	.326	477	18	2430	.248
Secondary roots	615	110	3850	.604	2650	47	4180	.998
1st side shoot leaves	675	700	2110	.448	1660	1400	910	.194
1st side stems	86	120	1820	.903				
2nd side shoot leaves	835	2350	2570	.439				
2nd side stems	81	110	1310	.090				

Woody stem = thick stem to 2 cm above soil surface. Stem base = thick stem from 2 cm abo

*) Leaves 1 + 2 of second stem.

†) Leaves of second stem.

III

xperiment

Manganese treated				Aluminium + Manganese treated				
Al ppm	Mn ppm	P ppm	Wt g	Al ppm	Mn ppm	P ppm	Wt g	
190	6150	4050	.033	2360	25000	5740	.250 *)	Leaf 1
				2200	25000	5860	.198	,, 1 + 2
98	13900	3210	.031					,, 2 + 3
				3330	31000	6600	.134 †)	,, 3
				3340	32000	5370	.249	,, 3 + 4
				2520	14500	5290	.036 †)	,, 4
510	17300	2690	.252					,, 4 + 5
				2740	15700	4920	.058	,, 5
				2420	11700	2100	.502 †)	,, 5 + 6
490	23600	2180	.297	2250	11000	2100	.617	,, 6 + 7
630	21800	1400	.058					,, 8
				3500	10500	2100	.312	,, 8 + 9
790	23400	1070	.248					,, 9
				4270	10200	1920	.155	,, 10
740	20700	1630	.684					,, 10 + 11
				8000	7900	1960	.028	,, 11
								,, 11 + 12
920	19300	1060	.741					,, 12 + 13
								,, 13
780	13800	815	.455					,, 14 + 15
								,, 16
1250	14200	800	.496					,, 16 + 17
								,, 17 + 18
1830	12500	762	.301					,, 18 + 19
2310	8400	630	.081					,, 20
								Flower buds
54	3100	960	.270	230	5100	5910	.228	Green stems
37	965	1650	1.180	566	3600	2250	.347	Brown stems
74	1930	825	.802	267	2100	2200	.403	Woody stems
55	1400	662	.178	408	2180	2540	.097	Stem bases
52	1550	650	.110	615	1800	3550	.076	Stems below
90	710	435	.840	340	2620	4700	.195	Root stocks
102	810	582	.467	1180	910	6010	.042	Primary roots
436	1820	2980	1.170	1310	1640	>6300	.462	Secondary roots
								1st side shoot
1150	15600	882	.067	3160	23000	1850	.088	{ leaves 1st side stems
								2nd side shoot leaves
								2nd side stems

soil surface. Stem below = thick stem from soil surface to 1 cm below.

TABLE IX

Summary of results of Kew pot experiment

Treatments: C = Control, A = Aluminium sulphate, M = Manganous sulphate, A + M = Aluminium and Manganous sulphates

		Aluminium ppm				Manganese ppm				Phosphorus ppm			
		C	A	M	A + M	C	A	M	A + M	C	A	M	A + M
Maxima	Leaves	2910	7500	2310	8000	3000	4650	23400	32000	2290	1400	4050	5800
Minima	Leaves	345	920	190	2200	575	840	6150	7900	1080	510	630	1920
Weighted means	Leaves	896	2100	956	2920	1440	2030	18200	17500	2100	1080	1280	3460
	Stems	64	235	50	385	100	60	1490	3360	1780	1650	1160	3060
	Roots	360	891	385	1030	64	41	3360	1800	3130	3241	3060	5840
Totals	Leaves	3116	10321	4219	7405	5018	9937	80084	44491	7306	5311	5644	8789
	Stems	146	653	133	443	227	166	3937	3609	4077	4559	3060	3525
	Roots	573	2968	625	721	102	137	2808	1260	4980	8010	4124	4078
Totals per Plant:													
C	Control		3835				5347				16363		
A:	Aluminium sulphate		13942				10240				17880		
M:	Manganous sulphate		4977				86829				12828		
A+M:	Manganous and Aluminium sulphates		8569				49360				16392		

Soils				
Treatment	C	A	M	A+M
pH	5.2	4.5	4.6	4.3
Avail. Al ppm	n.d.	120	30	84
Avail. Mn ppm	n.d.	tr.	970	868
Avail. P ppm	17	28	41	34

Additions to soil	
C:	nil
A:	284,000 ppm Aluminium
M:	674,000 ppm Manganese
A + M:	224,000 ppm Aluminium + 424,000 ppm Manganese

Dry weight of plant material				
	C	A	M	A+M
Tops	5.76	7.67	7.05	3.69
Underground	1.73	3.41	2.59	.78
Totals	7.49	11.08	9.64	4.47
Wt of soil, g	242	225	162	232

combined treatment obviously retarded growth and its high phosphate content was the result of uptake stimulated by the manganese. That toxic manganese is associated with the high phosphate uptake is shown in both stems of the A + M plant. The incipient chlorosis of the manganese treated plant was almost certainly due to an upset of the balance with iron. Aluminium did not prevent toxic symptoms and retarded growth in the A + M plant although the leaf lesions might not have formed if the plant had been kept shaded and the manganese applied in smaller doses over a longer period. Unlike manganese, aluminium is not translocated rapidly to the growing points but is accumulated gradually as the leaves age and lose their phosphate. Since the amount of available manganese in the soil of A + M plant was less than for the M plant and yet the manganese uptake was higher on a percentage basis in the former it is possible that the aluminium may have stimulated absorption of manganese.

The very strongly developed root stock of the aluminium treated plant may prove to be the most significant effect of this element as a good thick tap root or root-stock is most important as a reservoir of starch, without which the tea bush will not flourish and produce a crop.

EFFECT OF RAINFALL AND ELEVATION

Very wide ranges of rainfall figures and altitude are covered by the samples listed in Tables I–VII. It is apparent that aluminium uptake is not affected by either factor. Wherever the tea bush flourishes it will absorb aluminium from the soil and accumulate it in old leaves.

ALUMINIUM AND TEA SOILS

Most planters know that the tea bush will not thrive in limey soils and that the soil reaction has to be below about pH 6.0. All soils contain some calcium in an exchangeable form and soil of pH 6.0 would contain as much as ten milli-equivalents per cent if it were a clay and still tea would grow quite well. Calcium contents of the mature tea leaf are quite considerable (up to 7700 ppm, see also [17], [15]). It would appear that it is not the excess calcium which is the direct cause of tea failing in carbonate-free soils above pH

6.0 but the lack of something else. Aluminium does not occur in the soil solution in measurable amounts between pH 5.0 and pH 6.5 [25]) but owing to the tenacity with which tea roots hold soil particles it is conceivable that the root mucilage and soil colloids form one system in which cation exchange can take place. Exchangeable aluminium might well be the form which is available to the tea bush especially when the water-soluble form is absent or present in extremely small amounts. The first extractant to be tried for exchangeable aluminium was normal sodium chloride solution. This at once revealed abundant aluminium in all soils on which tea grew successfully and very little or none above pH 6.0. Since a considerable increase in soil acidity takes place with a neutral salt like sodium chloride to pH values well below that of the natural soil, the procedure of H e s l e p [16]) was tried, using normal calcium acetate buffered to approximately the same pH as the soil. The first set of results confirmed the sodium chloride data but owing to the difficulty in preparing a calcium acetate solution free from aluminium it was decided to use ammonium acetate buffered in the same way *). The original set of soils was extracted with these solutions and the results, although they are somewhat lower, all indicate that above pH 6.0 available aluminium is practically non-existent. These results are represented graphically in Figure 1.

Hut sites, old cattle kraals, termite mounds and ash accumulations are notoriously bad [9]) for tea cultivation on account of their high soil pH values (pH 6.4–8.2). No exchangeable aluminium is found in these soils.

In order to ascertain whether lack of aluminium was responsible for poor growth and yield on patches of soil that had long previously been hut sites, a series of carefully taken leaf samples were supplied by the Tea Research Institute of East Africa, Kericho. Ten flushes (2 leaves and a bud) were taken from each of five good and five adjacent unthrifty bushes. Composite surface soil samples were also examined. The analytical data for these samples are submitted in Table X. Despite the fact that the bushes were of the same height, growing on soil with a reaction well below the limit of pH 6.0, and differed only in lack of flushing ability of the "Hut"

*) Details of the method are given in the appendix.

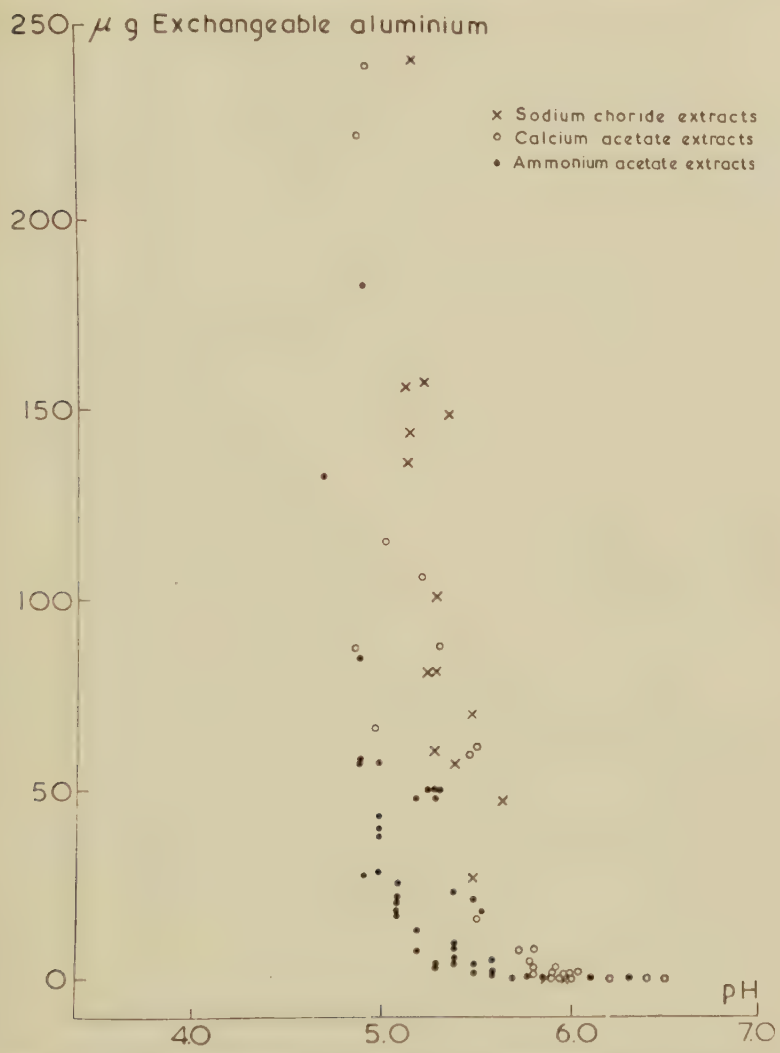

Fig. 1. The relationship of tea soil pH to exchangeable aluminium.

site bushes, there is a significant difference in the aluminium content of both the flushes and the soil, but not of the old leaves. The old leaves were all the fifth from the growing point but there may have been differences in age between them, which caused such wide variation within each set. Total aluminium in the leaves of the two sets of bushes is probably not very different as the flushes

would weigh very little. The low content of available soil aluminium may still be the cause of the poor tea on the hut site. If aluminium acts as a root stimulant as is suggested by the Kew pot experiment then a 14-fold difference in available aluminium might be the factor responsible. A large-scale pot experiment is now in progress at Kawanda Research Station in order to test this hypothesis.

TABLE X

Aluminium content of good and poor tea bushes

	Flushes *)		Old leaves	
	Good	Poor	Good	Poor
	400	217	3440	3040
	484	251	4000	3160
	419	285	3260	3880
	436	450	3890	3050
	350	256	5330	2350
Mean	418	292	3984	3096
Difference between means	126		888	
S.E. of differences	47		437	
L.S.D. for significance at				
P = 0.05,	107		1008	
P = 0.01,	156			

*) Ten flushes were taken off each bush but only one was used for analysis (selected at random). This was justified as no significant difference could be found between a single analysis and the mean of ten from the same bush.

Soil

	Good site	Hut site
Exchangeable aluminium (ppm) by NaCl	59	3.0
„ „ „ by $(CH_3COO)_2Ca$	27	0.0
„ „ „ by CH_3COONH_4	21	1.5

The old leaf data of Table X are not in accord with those of Parfenova and Troitskii[24]) who ascribe the differences of two patches of Georgian tea to the beneficial effects of aluminium in the better patch. From their soil profile descriptions it would appear that the prime reason for this difference would more feasibly be the waterlogging of the bad patch. Even on acid soil with a reaction well below pH 6.0, as in this instance, waterlogging is disasterous for tea bushes. The plants growing in this bad patch lacked the vigour and root development required for an uplift of sufficient water to convey, in the transpiration stream, much aluminium to the leaves.

ALUMINIUM AN ESSENTIAL ELEMENT FOR TEA

The invariable presence of large amounts of aluminium in the tea bush and of exchangeable aluminium in tea soils poses the question of essentiality. Aluminium has been demonstrated as being beneficial to water-plants by Stoklasa [33]), peas and millet by Sommer [32]), maize by Lipman [21]), solfatara plants by von Faber [14]), volcanic lava plants by Yoshii [37]) and ferns by Tauböck [35]). Only one aluminium-plant has been investigated in this connexion, viz. *Symplocos japonica* by Neger [23]). Aluminium was definitely proved to be essential but he did not prove that the accumulation of large amounts was essential or even beneficial. An interesting function of aluminium, for citrus at least, has been found by Liebig, Vanselow and Chapman [20]) in its preventing copper toxicity symptoms by a process of antagonism in the fine roots. Copper is not excluded nor prevented from migrating to the leaves but its toxicity is entirely nullified. The same effect may occur in tea but the present writer [7]) considered that manganese toxicity effects in the field might be precluded in a similar manner despite the results of the Kew pot experiment – Stocklasa had apparently the same opinion but did not try to demonstrate it. Manganese in excess of about 1,000 ppm of leaf dry weight induces toxic symptoms in most plants but the tea bush, in the field, can take it up to about 10,000 ppm and still remain healthy. The Kew pot plants appeared quite healthy until a large dose of manganous sulphate on a very hot day induced scorch or spotting in the youngest leaves of the plant treated with both manganese and aluminium; these leaves had accumulated 32,000 ppm manganese. Tubbs [36]) in a water culture experiment induced uptake to 15,400 ppm but his plants were defoliating within a week.

It is quite possible that aluminium confers no real benefit on the tea bush for Storey and Leach [34]) grew tea very successfully in water culture solutions under conditions in which very little, if any, aluminium uptake would occur.

The above suggests that a formal statistically controlled experiment is most desirable but time and facilities have not yet been available to the writer in Uganda. However, one simple preliminary experiment has been conducted and results were suf-

ficiently instructive to warrant recording here. This was an alkaline soil trial in the greenhouse at Kawanda Research Station.

ALKALINE SOIL EXPERIMENT

Nine large pots were filled with soil from a tea nursery in which the surviving plants were very stunted (only 6–12″ tall) at 18 months. This soil had a pH of 8.0 and this is probably the limit of soil alkalinity which the tea plant will tolerate. Four plants from this nursery were put into each pot and after they had been established about two months the leaves of two plants in each of three pots were brushed respectively with 1% solution of aluminium sulphate, ferrous sulphate and a 1 : 1 mixture of both. The treatment took place every day for two weeks, then sporadically over the next four weeks. The effect of the aluminium treatment was apparent in the change to patchy dark green of completely chlorotic young leaves. Injections with 1% aluminium and ferrous sulphates of chlorotic leaves on similar plants in the same soil produced green patches at the point of injection by aluminium sulphate within about four days. In only one instance among about 50 injections did the iron solution produce a green spot whereas the aluminium injections were about 70% effective.

After two years, only 2 aluminium, 1 iron and 4 aluminium and iron treated plants were still living, together with 7 controls, 2 of which were on the point of dying.

This experiment showed that aluminium does have some physiological effect on tea leaves but that it cannot, when applied in leaves make up for adverse soil conditions.

BUFFER INDEX AND ALUMINIUM UPTAKE

The failure of seedlings and eventually of mature bushes in alkaline soils and soils with a reaction above pH 6.0 indicates that a severe upset in the whole metabolism of the plant has taken place. This is certainly related to the buffer capacity or buffer index of the roots both in relation to iron chlorosis and aluminium uptake. In order to verify this, buffer index curves were prepared according to Small's method [30] [31] from titration – pH figures of 10 : 1 macerated water extracts of roots, stems and leaves from healthy

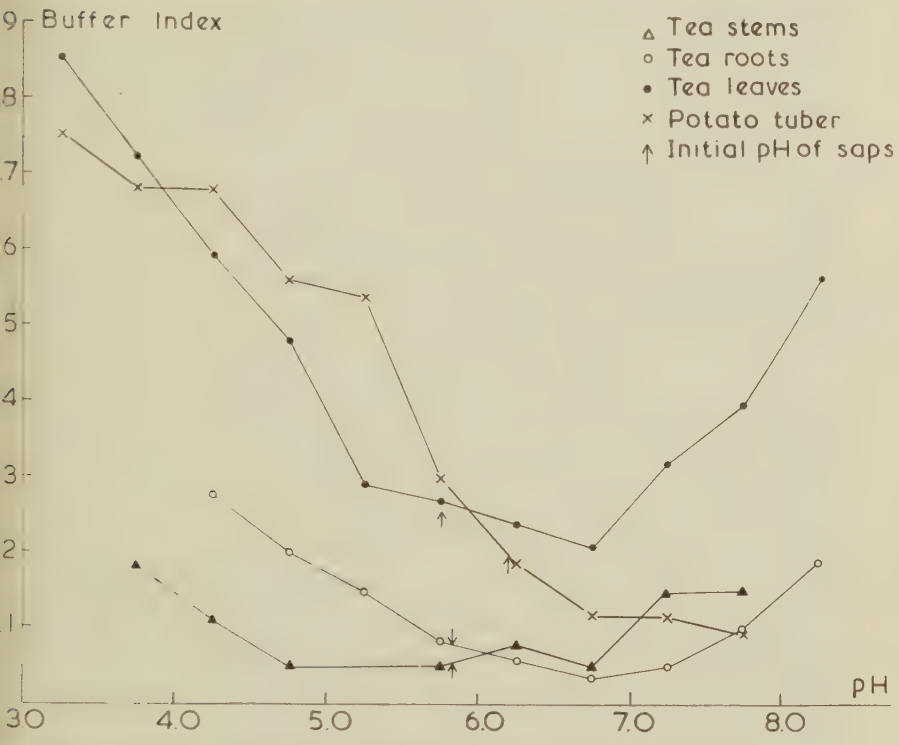

Fig. 2. Buffer index curves of tea bush sap compared with potato sap.

tea seedlings. Small used expressed sap for his determinations but as a press was not available a micromacerator was used and the results calculated on the basis of the sap present before dilution. In Figure 2 is a comparison curve for a potato tuber which is strongly buffered and this emphasises that the buffer capacities of the tea roots and stems are very low but it is somewhat higher in the leaves. This is manifest in the reaction of roots of tea plants grown in the alkaline pot experiment. These are pH 6.90 against pH 5.85 for healthy roots. Iron chlorosis usually occurs when the pH of root and stem tissue rises above pH 6.05. Aluminium intake is also severely restricted; the aluminium content of the leaves of 2 four-year old stunted plants in the alkaline pot experiment was only 42 ppm for a control and 89 ppm for a leaf that had been brushed with aluminium sulphate, and in a very chlorotic one-year old seedling growing in the same soil only 4.2 ppm.

In Ceylon the failure of tea on alkaline soils has long been associated with "bitten – off" disease. This condition is characterised by complete absence of tap root and few discoloured brittle lateral roots between the seed and the "bitten off" stump about one inch below. Internodes are short and leaves small and pale green. Since no pathogens could be found E d e n and G a d d [11]) ascribed the disease to soil alkalinity. Whether it is due to some upset in the buffer system of the roots, lack of iron or aluminium is not known but a waterculture experiment is now in progress at Kawanda to study this question.

THE TEA BUSH IN RELATION TO OTHER ALUMINIUM-PLANTS

Now that tea has been shown to be such a constant accumulator of aluminium, even if it be luxury consumption, it should be interesting to examine the position of the tea plant in this respect with the rest of the plant world. The tribe *Gordonieae* to which the tea bush belongs and the tribe *Ternstroemieae* are almost exclusively aluminium accumulators and this feature is not shown in other members of the *Theaceae*. A i r y–S h a w [1]) on morphological grounds has advocated the separating of these tribes into distinct families or else include them with another family which is placed in present taxonomic systems about as far as possible from the *Theaceae*. This family is the *Symplocaceae* one of the strongest of all aluminium-plant families, with aluminium contents rising to 70,000 ppm. This relationship has been cited as one of the best examples of reticulate affinity [12]).

Evidence has been adduced [7]) to show that aluminium-plants are primitive; some are indeed, among the most primitive of all plants in their respective classes, *e.g. Andraeaceae* of the mosses and *Marattiaceae* among the ferns. Fossil records of the aluminium-accumulating fern families *Gleicheniaceae, Matoniaceae* and *Dipteraceae* show that these tropical ferns once extended far over Asia, even to Greenland. Today they are restricted to the wettest tropics and the last two families to a small corner of S.E. Asia. Reduction in area covered by plants is related to degree of senescence – such plants if not living fossils are relict forms. Among the flowering aluminium-plants there are several which are definitely on the decline; one, at least, (*Shortia galacifolia*) was thought extinct

in the wild state for a considerable time. In this connexion it is interesting to speculate on the status of the tea plant because truly wild specimens have rarely been collected.

According to Sealy [29]) no authentic wild bushes of the broad-leaved Indian variety *assamica* have yet been found despite diligent searching by Kingdon Ward [19]). But the narrow-leaved Chinese variety *sinensis* was discovered by Henry in virgin forests of South Yunnan and by Forrest in West Yunnan. Virgin forests described by collectors have often subsequently proved to be secondary; it would be instructive to have a modern ecologist's opinion on these Yunnan forests. It is not beyond the bounds of possibility that the tea bush, like the Ginkgo, owes its survival to cultivation and that its existence would have been precarious if its stimulating properties had not been discovered.

SUMMARY

The phenomenon of uptake of aluminium by the tea bush has been examined in relation to its constancy as a characteristic feature, age of leaf and tree, genetic constitution, resistance to certain diseases, distribution within the plant, interactions with manganese and phosphorus, soil, essentiality and finally in relation to other aluminium-plants.

Strong aluminium absorption appears to be a constant feature for all healthy bushes of any age, the element is stored in the oldest leaves but it does not impart any resistance to "blister blight" but it occurs to a greater extent than normal in flushes with "tea yellows"; it is gene-controlled, there being three distinct levels of accumulation corresponding with the three major divisions of the species. The presence of abundant available aluminium in the soil will not prevent excessive uptake of manganese accompanied by severe leaf scorch and spotting in bright light. Aluminium tends to diminish leaf phosphorus while manganese tends to increase it. Large amounts of available soil manganese may induce greater uptake of aluminium and *vice versa*. Small quantities of aluminium within tea leaves are associated with degree of greenness, but the large accumulations probably do not serve any useful purpose. Exchangeable soil aluminium may stimulate roots, particularly tap-roots or root-stocks. The tea bush may be a relict plant like so many of other aluminium accumulators.

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REFERENCES

1) Airy-Shaw, H. K. *Symplococarpon hintoni* (Bullock) Airy-Shaw. Icones Plantarum t3342 (1937).
2) Bertrand, G. et Levy, G., La teneur des plantes, notamment des plantes alimentaires en aluminium. Compt. Rend. Acad. Sci. Paris **192**, 525 (1931).
3) Chenery, E. M., The problem of the blue hydrangea. J. Roy. Hort. Soc. **62**, 304 (1937).
4) Chenery, E. M., Aluminium in the plant world, part I, general survey in dicotyledons. Kew Bulletin, 173 (1948).
5) Chenery, E. M., Aluminium in plants and its relation to plant pigments. Ann. Botany N.S. **12**, 121 (1948).
6) Chenery, E. M., Aluminium in the plant world, parts II and III, monocotyledons, gymnosperms and cryptogams. Kew Bulletin, 463 (1949).
7) Chenery, E. M., Contributions to the biogeochemistry of aluminium 1948–50. Colonial Office Mimeograph C. 0/1529/51 (1951).
8) Child, R., Tea and soil acidity. Tea Research Inst. E. Africa Ann. Rept. **23** (1951).
9) Child, R., The selection of soils suitable for tea. Tea Research Inst. E. Africa, Pam. 5, 6 (1952).
10) Eden, T., Private communication, 1949.
11) Eden, T. and Gadd, C. H., Reports of agricultural chemist and plant pathologist for 1931. Tea Research Inst. Ceylon Bull **8**, 37 (1932).
12) Editorial, Expansion of plant systematics. Nature **158**, 535 (1946).
13) von Faber, F. C., Die Kraterpflanzen Javas in physiologisch-ökologischer Beziehung. Arbt. Treub-Laboratorium, Buitenzorg **1**, 1 (1927).
14) von Faber, F. C., Untersuchungen über die Physiologie der javanischen Solfataren-Pflanzen. Flora **118**, 89 (1925).
15) Hallier, H., Beiträge zur Kenntnis der *Linaceae* (DC. 1819) Dumort. Botan. Zentralblatt. Beih. **39**, 128 (1922).
16) Heslep, J. M., A study of the infertility of two acid soils. Soil Sci. **72**, 67 (1951).
17) Hutchinson, G. E., The biogeochemistry of aluminium and of certain related elements. Quart. Rev. Biol. **18**, 1 (1943).
18) Kingdon-Ward, F., Does wild tea exist? Nature **165**, 297 (1950).
19) Lamb, J., Private communication, 1952.
20) Liebig, G. F., Vanselow, A. P. and Chapman, H. D., Effects of aluminium on copper toxicity as revealed by solution-culture and spectrographic studies of citrus. Soil Sci. **53**, 341 (1942).
21) Lipman, C. B., Importance of silicon, aluminium and chlorine for higher plants. Soil Sci. **45**, 189 (1938).
22) McMurtrey, J. E. and Robinson, W. O., Neglected soil constituents that affect plant and animal development. Soils and Men, U.S.D.A. Yearbook, 813 (1938).

23) Neger, F. W., Neue Methoden und Ergebnisse der Microchemie der Pflanzen. Flora **116**, 323 (1923).
24) Parfenova, E. I. and Troitskii, A. I., Possible causes of a failure of tea bushes. Pochvovedenie, 322 (1951). (Translation by Bureau Inter-africain des Sols, Paris).
25) Pierre, W. H., Hydrogen ion concentration, aluminium concentration in the soil solution and percentage base saturation as factors affecting plant growth on acid soils. Soil Sci. **31**, 183 (1931).
26) Polynov, B. B., The red crust of weathering and its soils. Pochvovedenie 7 (1944).
27) Polynov, B. B., Leading ideas of the contemporary study of soil formation and development. Pochvovedenie, 3 (1948). (Refs. 26, 27, translated by Commonwealth Bureau of Soil Science).
28) Robinson, W. O., The agricultural significance of the minor elements. Am. Fertilizer **89**, No. 8, 9 (1938). (for private communication by K. C. Hou).
29) Sealy, J. R., Private communication (1953).
30) Small, J., pH and plants. Bailliere Tindall and Cox, London, 1946.
31) Small, J. and Jackson, T., Relative buffer-index values for root saps of some crop plants. J. Agr. Sci. **38**, 343 (1948).
32) Sommer, A. L., Studies concerning the essential nature of aluminium and silicon for plant growth. Univ. Calif. Publs. Agr. Sci. **5**, 57 (1926).
33) Stoklasa, J., Über die Verbreitung des Aluminiums in der Natur. Jena, G. Fischer, 1922.
34) Storey, H. H. and Leach, R., A sulphur-deficiency disease of the tea bush. Ann. Applied Biol. **20**, 23 (1933).
35) Tauböck, K., Über die Lebensnotwendigkeit des Aluminiums für Pteridophyten. Botan. Arch **43**, 219 (1942). (Translation by Bureau Inter-africain des Sols, Paris).
36) Tubbs, F. R., Manganese in tea. Tea Research Inst. Ceylon Ann. Rept. 1933. Bull. 11, 36 (1934).
37) Yoshii, Y., Some experiments on the action of aluminium on plants. Sci. Rept. Tôhoku Imp. Univ. (ser. 4), **3**, 547 (1928).
38) Yoshii, Y. und Jimbo, T., Mikrochemischer Nachweis von Aluminium und sein Vorkommen im Pflanzenreiche. Sci. Rep. Tôhoku Imp. Univ. (ser. 4), **7**, 65 (1932).

APPENDIX

METHODS OF ANALYSIS

Exchangeable aluminium using sodium chloride or calcium acetate

Preparation of samples. About 1 g soil (air dry, 2 mm sieved) is accurately weighed out (to .01 g) into a folded filter-paper, the paper with soil is placed in a funnel inserted in a 100 ml measuring flask; distilled water is added and the soil leached until the 100-ml mark is reached. The leachate contains water-soluble Al which is not detectible above pH 4.3.

The funnel (and its contents) is then inserted into another 100 ml measuring flask and the soil leached with 100 ml 1 Molar NaCl. The leachate contains practically all the exchangeable Al for soils above

pH 5.0, a second leaching with 100 ml NaCl may be necessary for more acid clayey soils.

The leachates should be perfectly clear and colourless.

Normal calcium acetate extracts are prepared in the same way.

Reagents *). Aluminon reagent — Ammonium aurine tricarboxylate, 0.75 g; gum acacia, 15 g; ammonium acetate, 200 g; concentrated hydrochloric acid (A.R.), 189 ml; dissolved separately, mixed, filtered and made up to 1500 ml

Thioglycollic acid — One ml diluted to 100 ml.

Aluminium sulphate standard — Stock solution containing 250 ppm of aluminium and containing 4 ml of concentrated nitric acid per litre. Ten ml of this diluted to 250 ml gave the 10-μg aluminium standard, aliquots of which were used in preparing the curves. The stock solution must be analysed gravimetrically for aluminium, especially in the tropics, where loss of water of crystallisation may lead to errors.

Development of red aluminium lakes

2 ml are pipetted into 25 ml numbered measuring flasks, 1 ml (1 : 50 v/v) HNO_3 added to each, then 1 ml (1% thioglycollic acid) and then 5 ml Aluminon reagent. The contents of the flasks are mixed by swirling and made up to the mark. A batch of 15 are prepared at a time and the flasks placed in a perforated zinc basket (perforations 1 cm) 25 cm diam. taking care to wedge them up with test-tubes or lead piping to prevent spilling. The zinc basket is then dropped carefully into a sauce-pan of vigorously boiling water (heated with 2 bunsens) and heated for 15 minutes (the water usually takes about 2 minutes to come to the boil again and should be kept gently boiling for the next 13 minutes). On taking out of the water-bath the flasks are removed from the basket and stood in a shady place to cool down to room temperature. The cooling should last at least 6 hours. So long as all determinations are made at approximately the same temperature, ($\pm$ 2°C) the actual temperature is not important. The coloured solutions are compared in a photo-electric colorimeter at a *fixed* time between 6 and 30 hours after taking out of the boiling water bath. Rapid cooling under the tap is also feasible but the solutions should be stood for at least an hour at more or less constant temperature afterwards.

Curves are constructed from the standard Al solutions plus 2 ml of the extractant with a range of 0 to 55 mg Al per 25 ml in exactly the above manner, and the concentration of the unknowns read off directly.

Exchangeable aluminium with ammonium acetate as extractant

10 grams of soil are weighed into 250-ml conical flasks and 50 ml of ammonium acetate added to each. The ammonium acetate has the same pH ($\pm$ .05) as the soil. The contents of the flasks are swirled, allowed to stand overnight, swirled and filtered into a dry flask next morning. Two ml of the leachate are used as above to determine aluminium without previ-

*) E. M. Chenery, Analyst **73**, 501 (1948).

ously making up the final volume up to 50 ml as equilibrium is completed in the original conical flasks. Quantities of aluminium up to 1 ppm on the soil basis may be determined directly in this way. For soils with less than .5 ppm exchangeable aluminium, 25 ml of the ammonium acetate leachates are pipetted into silica dishes or crucibles and evaporated to dryness overnight. The dishes are then placed in the electric muffle and maintained at 450–500°C for about 6 hours. Ten drops of conc. HNO_3 are carefully added to the residues and then 1 drop of 100 volume H_2O_2. The acid is carefully driven off on a low temperature hot plate and heated until none can be detected by smell. 1 ml of .5% thioglycollic acid (or $\frac{1}{2}$ ml of 1%) are added to each dish which are then warmed for a few minutes, 2 ml of N ammonium acetate at pH 7.0 are added followed by 2 ml of aluminon reagent. The whole is well stirred and transferred to a test-tube graduated at 10 ml and made up to the mark. The red aluminium colour is developed in the usual way. If the soil contains between .5 and 2.0 ppm the above procedure is used but all quantities are multiplied $2\frac{1}{2}$ times and the colour developed in 25 ml graduated flasks.

Standard curves are constructed using 2 ml of *N* ammonium acetate at pH 7.0 in each standard.

Total aluminium in plants

The cleanest possible material is selected and gently rubbed with a swab of cotton wool soaked in 50% alcohol, acidified to about 1% with HNO_3. It is then shaken vigorously 3 times with the warm acidified alcohol in a test-tube or covered beaker. After being placed on a clean filter-paper with platinum-tipped forceps it is securely folded in the paper and dried for 3–6 hours at 105°C if the material was originally air-dry; if fresh 24 hours drying is necessary.

The leaves or roots may then be crushed in the filter-paper to very fine particles without coming into contact with the hands or they may be ground in an agate mortar (reserved for plants only). A mechanical grinder is undesirable as the risk of contamination is too great.

100 to 200 mg of the clean, oven-dry material from non-aluminium accumulating species or 3 to 15 mg of accumulator-plant leaves, are weighed into tared platinum crucibles (1 or 5 ml). A batch of 6 is a convenient number to handle at one time. The crucibles are covered with lids (9/10 coverage) and placed over bunsens adjusted to the lowest possible flame or in an electric furnace, at 400 to 500°C. When all the volatile matter has been driven off the flames are raised very slightly but under no circumstances are they allowed to come into contact with the crucibles nor are these allowed to show the slightest sign of reddening. A small sample takes 3 to 10 minutes and a larger one up to 1 hour to become completely ashed. If an electric furnace is used the final temperature should be set at 500°C.

To the cooled ashes are added 1 drop conc. HNO_3 and 1 drop 5 vol. % H_2O_2 taking care that the crucibles are held in a slant-wise position to avoid spattering. They are then similarly placed in a sand-bath at about

200°C and evaporated to dryness. If much manganese is present the ashes are pale brown to almost black in colour and visible detection of small unburnt carbon particles is impossible. A second gentle heating for 2–3 minutes followed by the above treatment usually suffices to complete the ashing. The final acid solution must be absolutely clear and colourless; rarely are more than 2 evaporations necessary to ensure this. Care should be taken to see that any ash adhering to the sides of the crucibles is attacked by the acid and peroxide.

When the final traces of HNO_3 have been driven off (no detectible smell), the crucibles are cooled slightly and 1 ml 1 : 50 HNO_3 added to each, together with 1–2 ml water. They are heated on the sand-bath for a further 10 minutes and their contents then transferred to the 25 ml measuring flasks. If silica particles are present, they must be filtered off during this transference. Silica always occurs in graminaceous species, beech trees and a few other broad leaved trees but very rarely in aluminium-accumulators.

1 ml 1% thioglycollic acid and 5 ml Aluminon reagent are added to each flask and the red aluminium lake developed as before.

It is often desirable to determine Fe, Mn and P on the same ash extract, in such cases the minimum quantity of leaf material is raised to 50 mg and aliquots taken from the ash solutions previously made up to 25 ml.

An accuracy of better than $\pm$ 1% error can be obtained by these methods and a general working error of $\pm$ 2% of the actual Al present.

Note. If the platinum crucibles are allowed to show signs of reddening the aluminium is rendered insoluble and can only be estimated by a sodium carbonate fusion.

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THE EFFECTS AND INTERACTIONS OF MOLYBDENUM, LIME AND PHOSPHATE TREATMENTS ON THE YIELD AND COMPOSITION OF WHITE CLOVER, GROWN ON ACID, MOLYBDENUM RESPONSIVE SOILS

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INTRODUCTION

Molybdenum deficiency has been shown to be a highly significant factor in pasture production in Australia and New Zealand. Anderson and others in Australia (Anderson [2]), Anderson and Thomas [7]), Anderson and Oertel [8])) have demonstrated the importance of molybdenum for the satisfactory growth of legumes (mainly subterranean clover, *Trifolium subterraneum*, and lucerne, *Medicago sativa*) on several different soils. Marked responses in the growth of legumes to applications of molybdenum have also been noted in New Zealand by Lobb [17]), Davies, Holmes and Lynch [11]) and Adams [1]).

One of the most significant facts emerging in many of these experiments is the marked negative lime-molybdenum interaction, and it would appear that one of the main functions of lime on some soils is to make molybdenum more available to plants. On some acid soils dressings of less than 2 ounces of sodium molybdate per acre have given increases in pasture growth comparable with the application of one or more tons of ground limestone per acre. Responses of legumes to molybdenum have been noted particularly because of its essential role in nitrogen fixation as distinct from the smaller need in the host-plant metabolism, but responses have also been noted in many other crops.

The significance of the lime-molybdenum interaction in relation

to the classical concepts of the "lime requirement" of soils and so-called "phosphate fixation" on acid soils has been briefly discussed by Walker [2]). Anderson and McLachlan [5]) have also drawn attention to the fact that much of the evidence in the literature on phosphate fixation has been obtained from laboratory studies of the soil, or from short-term field and pot trials, and as a result of Australian work on molybdenum, sulphur, copper and zinc deficiencies, they state that a number of soil fertility problems once thought to be due to intense phosphate fixation are now known to be due primarily to deficiencies of other nutrients.

In view of the importance of white clover in New Zealand pastoral farming, it was decided to examine the effect of lime-molybdenum-phosphate interactions on the yields of dry matter, and to analyse the clover for N, Ca, P, Mo and Mn, in order to study more closely some of the factors involved in the soil-acidity complex.

EXPERIMENTAL

Lay-out and treatments

3 × 3 × 3 factorial designs of single replication were employed on two soils, using small waxed honey cartons holding 750 g soil, under glasshouse conditions. The following acre rates were calculated on the weight of soil basis:

(1) Calcium hydroxide equivalent to 0, 1½ and 3 tons $CaCO_3$ per acre (L_0, L_1 and L_2).

(2) Monocalcium phosphate at 0, 0.33 and 0.66 cwt P_2O_5 per acre (P_0, P_1 and P_2).

(3) Sodium molybdate at 0, 1 and 2 oz per acre (M_0, M_1 and M_2).

The lime and phosphate were mixed throughout the bulk of soil and the sodium molybdate was sprayed on the surface of the pots. Seeds of New Zealand certified nucleus stock white clover seed were sown and eventually thinned to 3 plants per pot. The pots were weighed and watered regularly to 70 per cent of the water-holding capacity. The experiment was started in January 1953 and the clovers harvested on two occasions in the winter (June) and spring (October).

Soils

Two acid soils taken from untreated areas on pastures known to have given marked responses to molybdenum were kindly supplied to us by Mr. W. R. Lobb of North Otago, New Zealand. The soils were silt loams formed from similar parent material (greywacke loess), the major difference in soil-forming factors being that of rainfall which is 30" or

more in the soil of Trial 2, and 25" in Trial 1. The natural cover was tussock grassland and sown pastures revert to dominant brown-top (*Agrostis tenuis*). Relevant analytical data are shown in Table I.

TABLE I

	pH	Total exch. capacity me %	Exch. Ca me %	Truog P mg %	Exch.Mn ppm	Easily reducible Mn ppm	Exch. + easily reducible Mn	Avail. Mo ppm	N %	C %	C/N
1	5.3	18.2	4.5	2.9	22	28	50	0.05	0.23	3.76	16.3
2	5.3	19.8	4.0	3.8	79	117	196	0.08	0.29	4.70	16.2

Points of note are the low pH and exchangeable Ca. The Truog [26]) phosphate levels would be classified in New Zealand as medium in both cases. The main differences between the two soils are the higher available molybdenum [13]) and higher exchangeable and easily reducible Mn [16]) of Trial 2 compared with Trial 1.

The "lime requirement" of both soils estimated by the buffer-curve technique to bring the pH to 6.5 was approximately 3 tons $CaCO_3$ per acre, and the quantities used in the trials represent therefore 0, half and full ' lime requirement" rates. The actual pH levels at the different rates of liming were as follows:

	Trial 1	Trial 2
L_0	5.3	5.3
L_1	5.8	5.7
L_2	6.4	6.2

Conventional methods were used for the analysis of the plant material. Manganese was determined using periodate oxidation and molybdenum by the thiocyanate method using amyl alcohol as colour extractant.

RESULTS

To reduce the tabular data to a minimum, only selected data will be presented to illustrate points under discussion, but the means of the totals, and two-way tables for yields of dry matter as used for statistical analysis with standard errors are presented in the appendix (Table VII).

Yields of dry matter

The lime-molybdenum interaction. Lime gave highly significant increases in yield on both soils for both cuts. For the first cuts the full L.R. rates gave significantly higher yields than the half rates, whereas for the second cuts, although lime still gave highly significant responses, the main effects for both rates of

lime were almost identical. The lime-molybdenum interactions are shown in Fig. 1 and illustrate in a striking fashion the change from a positive interaction for the first cut to a negative interaction

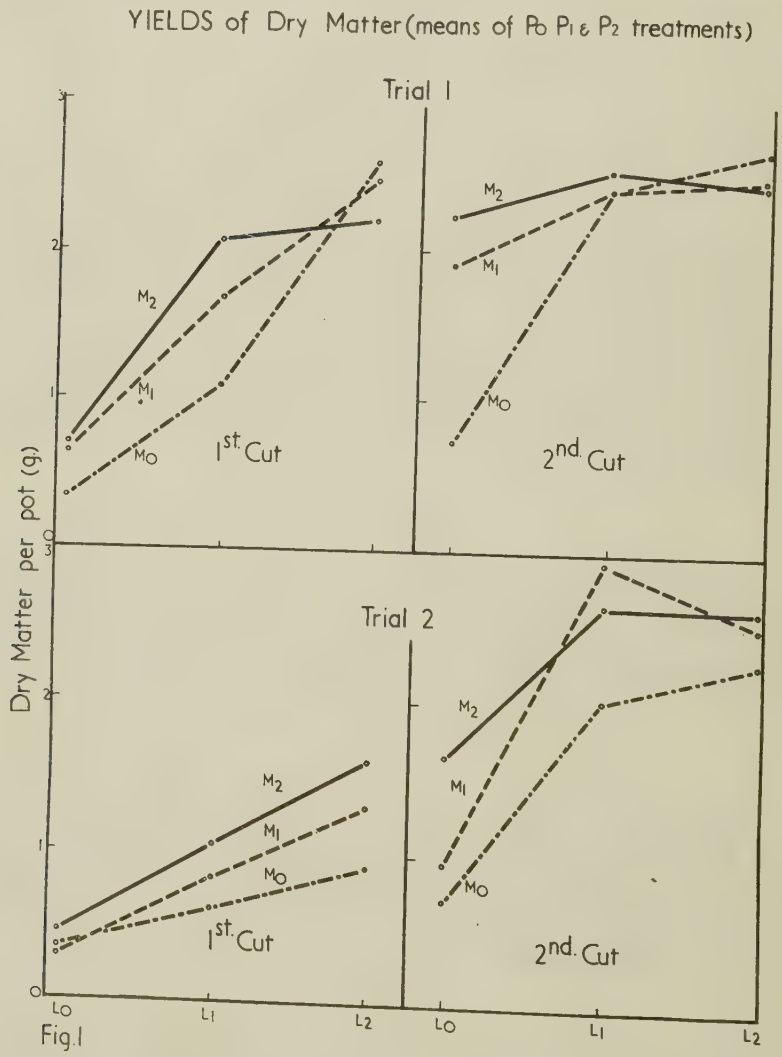

Fig.1

at the second cut. The very small response to molybdenum in the absence of lime for the first cuts was completely at variance

with the very marked responses to molybdenum on established pastures on these two soils and it was only at the second cut that large responses were obtained from molybdenum in the absence of lime.

The most likely explanation of these results is one advanced by Anderson and Moye[6]) and Spencer[24]). These authors have shown with subterranean clover on soils below pH 5 (and possibly below 5.5) that nodulation may be defective for some considerable time (up to two years). Subsequently, nodulation improves and the response to molybdenum increases progressively; as little as 2 cwt per acre $CaCO_3$ drilled with the seed improves nodulation, and molybdenum then gives immediate responses, with yields quite as high as those obtained from much heavier dressings of lime. It has been shown that both an increase in pH and calcium are needed to effect rapid nodulation, and it would appear that molybdenum itself does not stimulate nodulation, but stimulates symbiotic N-fixation after the nodules are already formed.

It was not possible to check the nodulation of the clovers in these trials until the end of the experiment by which time the clovers in all treatments were well nodulated. It would seem that white clover behaves in a manner similar to sub clover. Nodules may only slowly develop on these acid soils in the absence of lime, and molybdenum had little effect initially on clover growth.

In the case of the first cut in Trial 1, molybdenum gave a marked response at L_1 presumably because nodulation had been stimulated, whereas at L_2, this heavier dressing of lime not only stimulated nodulation but also released sufficient native molybdenum from the soil so that added molybdenum gave no further increase in yield. Subsequently nodulation may have improved on the unlimed soil and for the second cut molybdenum gave big responses and the yield at L_0M_2 was almost as high as the yields from the L_2 dressings.

In Trial 2 for the first cut increasing responses to molybdenum were obtained with increasing rates of lime, whereas for the second cut molybdenum gave the biggest responses in the absence of lime, with a general tendency for the response to fall as the amount of lime was increased — an exactly opposite effect in the two cuts. Whilst the general pattern of the two trials is similar, it will be shown later in the case of Trial 2 that manganese toxicity and a

Mn–Mo antagonism, rather than straight molybdenum deficiency were probably responsible for the poor clover growth at L_0M_0. The highest rate of molybdenum in the absence of lime was not sufficient to give maximum yields of dry matter in the second cut of Trial 2, but would appear to have been almost adequate in Trial 1.

The responses to P as affected by lime and molybdenum. The responses to P were significant ($P = .05$) for the second cut of Trial 1, and for both cuts ($P = .01$) in Trial 2. The mean response to P $\left(\text{taken as } \frac{P_1 + P_2 - 2P_0}{2}\right)$ was calculated

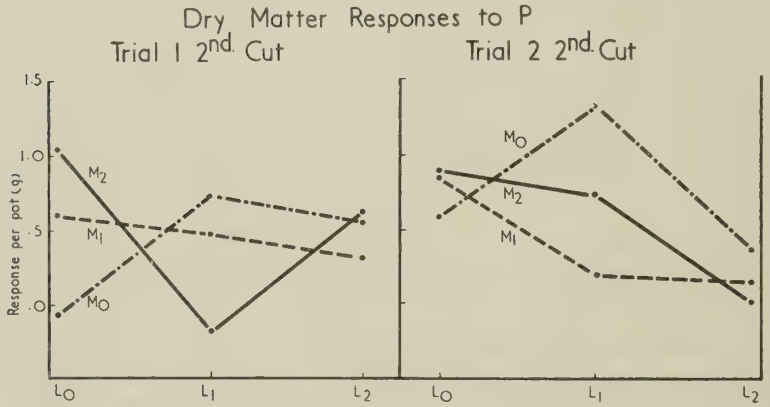

Fig. 2

for each combination of lime and molybdenum and the results are plotted in Fig. 2. As the same trends were apparent for both cuts the data for the second cut only are shown. In the case of Trial 1, at M_0, phosphate has given no response in the absence of lime, a big response at L_1 and a slightly smaller response at L_2. This could lead to the assumption that phosphate is more readily fixed in more unavailable forms on the unlimed soil and therefore gives smaller responses. When molybdenum is applied, however, at L_0, P gives increased responses with increasing applications of molybdenum, higher in fact than on the limed soils. This latter effect may be due to the lime rendering native P more available, so that response to applied P is accordingly lower. There is some evidence (Stout, Meagher *et al.* [25]) that calcium mono-

phosphate may liberate molybdenum by exchanging phosphate for adsorbed molybdate, and the higher response to P at L_0M_2 could possibly be due to an exchange of this nature, although the same effect should have been noted at L_0M_0. Unfortunately it was necessary to bulk the P treatments for molybdenum analyses so this point could not be checked. The same general trends were also shown in Trial 2.

Content and yields of N, Ca, P, Mo and Mn

Individual treatments were analysed separately for these constituents but for simplicity in this section Tables II–VI show only the mean yields of the P-treatments and weighted mean content of these same treatments, either as percentages or ppm.

(*a*) Nitrogen. The yields and per cent N have been condensed in Table II.

TABLE II

	Yield of N (mg per pot) *) and % N **) in dry matter					
	Trial 1					
	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	10(2.95)	29(2.61)	65(2.61)	19(2.57)	62(2.57)	67(2.50)
M_1	21(3.22)	46(2.75)	64(2.75)	51(2.72)	75(3.10)	67(2.69)
M_2	23(3.19)	57(2.78)	58(2.78)	60(2.68)	72(2.84)	70(2.84)
	Trial 2					
	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	6(1.66)	14(2.16)	20(2.20)	16(2.22)	46(2.24)	45(1.97)
M_1	5(1.55)	22(2.66)	35(2.72)	15(1.62)	65(2.20)	63(2.49)
M_2	9(2.08)	33(3.14)	45(2.41)	41(2.49)	67(2.48)	65(2.47)

*) Mean of yields from P_0 P_1 P_2 treatments.

**) In brackets after yield data — weighted means for P_0 P_1 P_2 treatments.

The effect of lime and molybdenum on yields of N are similar to the effects on the yield of dry matter. By the second cut, M_2 in the absence of lime was yielding almost as much N as the lime treatments in the absence of molybdenum. The per cent N in the clovers is very low by New Zealand standards (usually 3–4 per cent), especially as signs of N-deficiency were only usually evident on the treatments getting no lime or no molybdenum. The most acute

symptoms of nitrogen deficiency were for the first cut in Trial 2 in all the L_0 and M_0 pots, and these contained the lowest per cent N. Whilst there are no consistent trends the per cent N may be said to increase slightly with applications of lime and molybdenum.

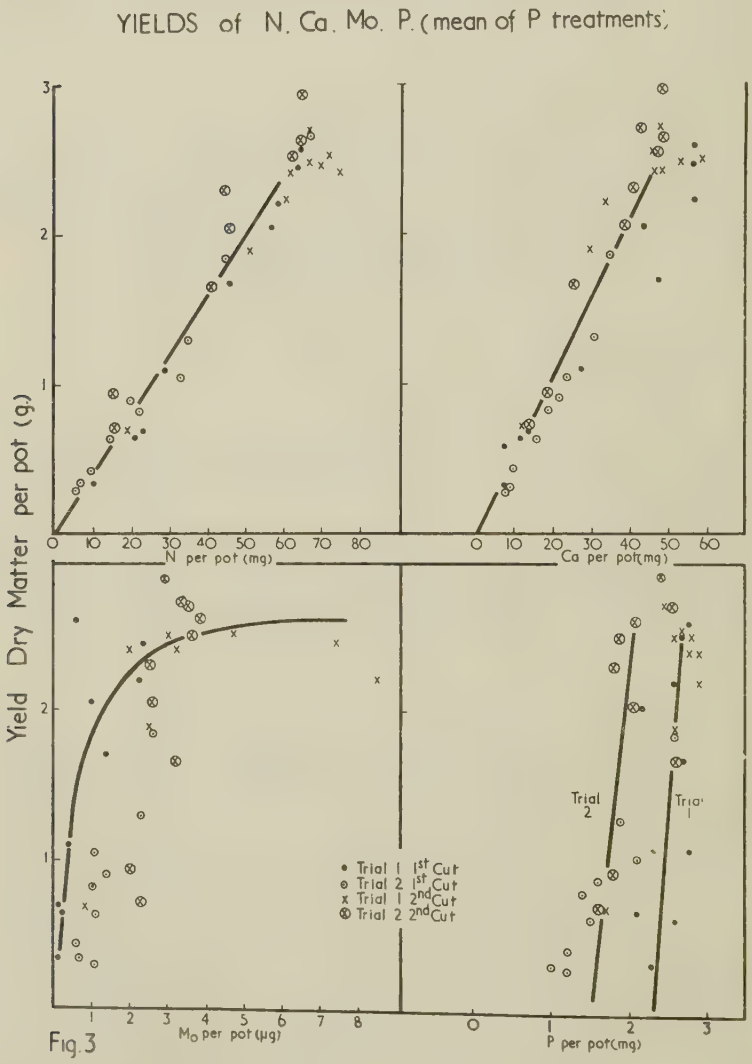
YIELDS of N. Ca. Mo. P. (mean of P treatments)
Yield Dry Matter per pot (g.)
N per pot (mg)
Ca per pot(mg)
Mo per pot(µg)
P per pot(mg)
Trial 2
Trial 1
• Trial 1 1st Cut
⊙ Trial 2 1st Cut
x Trial 1 2nd Cut
⊗ Trial 2 2nd Cut

Fig. 3

The remarkable constancy of the per cent N with the varying lime and molybdenum treatments is shown in Fig. 3 where the

yields of N for the separate cuts for both trials are plotted against the yields of dry matter. This linear relationship shows almost without question that the effect of lime and molybdenum separately and together is to increase the symbiotic fixation of nitrogen, the low supply of which was responsible for the poor growth of the clover. The lime may have increased the amount of mineral N formed from the soil organic matter and this would be additional to any effect on increased availability of molybdenum. (The soils incubated alone for five weeks at 30°C and 50% W.H.C. contained the same amount of mineral N as the soils incubated with Mo, and slightly less than when incubated with the addition of lime.)

The slope of the line coincides approximately with a nitrogen content of 2.5 per cent N, or in other words, 1 mg of N has given an increase in yield of 40 mg of dry matter. It is tempting to think that all the nitrogen up to a level of 2.5 per cent was involved in the photosynthetic processes, conditions for which were presumably good under the particular conditions of these trials. The slight tendency for the curve to flatten at the highest yield may indicate that at this point carbohydrate synthesis became the limiting factor rather than the amount of N fixed, and there was consequently a tendency for the per cent N to increase.

TABLE III

Yield of Ca (mg per pot) and % Ca in dry matter

Trial 1

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	8(2.33)	28(2.55)	57(2.20)	12(1.71)	47(1.95)	48(1.77)
M_1	12(1.93)	43(2.56)	57(2.33)	30(1.57)	48(1.98)	58(2.34)
M_2	14(2.06)	44(2.17)	57(2.59)	33(1.47)	46(1.83)	53(2.18)

Trial 2

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	9(2.70)	16(2.57)	22(2.40)	14(1.90)	39(1.90)	41(1.78)
M_1	8(2.60)	19(2.33)	31(2.35)	19(2.02)	48(1.64)	47(1.87)
M_2	10(2.30)	24(2.32)	35(1.91)	26(1.58)	43(1.62)	49(1.86)

(*b*) Calcium. The data for calcium are shown in Table III. In both trials the per cent Ca was higher in the first than the second cuts but it is commonly found in clovers that the per cent

Ca is higher in autumn than in spring [29]). Applications of lime have by no means consistently increased the per cent Ca especially in Trial 2. This is perhaps to be expected if the main effect of lime has been due not to the correction of Ca-deficiency but to the correction of other deficiencies (Mo) or excesses (Mn), thus allowing greater fixation of N which gives higher yields of dry matter. The data of particular interest are those for the L_0M_2, L_1M_0 and L_2M_0 treatments. In all cases for both cuts molybdenum in the absence of lime has given appreciably lower Ca-contents than lime in the absence of molybdenum.

The yields of Ca are plotted against dry matter in Fig. 3. The linear relationship is not quite as clear cut as in the case of N, but there is obviously a marked correlation between the N-uptake and Ca-uptake. Ca-uptake has been dependent chiefly on N-fixation; the overall average Ca-content is 2.0% and the N:Ca ratio (equivalents) of the uptakes is approximately 1.8. The wider scatter of points at the higher yields is due to the lower per cent Ca for the Mo-treated clovers, and the higher per cent Ca for the lime-treated clovers.

TABLE IV

Yields of P (mg per pot) and % P in dry matter

Trial 1

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	2.3(.68)	2.8(.25)	2.8(.11)	1.7(.23)	2.9(.12)	2.2(.09)
M_1	2.6(.41)	2.7(.16)	2.7(.11)	2.6(.14)	2.8(.11)	2.8(.11)
M_2	2.1(.30)	2.2(.11)	2.6(.12)	2.9(.13)	2.6(.10)	2.5(.10)

Trial 2

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	1.0(.28)	1.5(.24)	1.6(.18)	1.6(.22)	2.1(.10)	1.8(.08)
M_1	1.2(.36)	1.4(.18)	1.9(.15)	1.8(.19)	2.4(.08)	1.9(.08)
M_2	1.2(.29)	2.1(.20)	2.6(.14)	2.6(.16)	2.5(.09)	2.1(.08)

(*c*) Phosphorus. These data are presented in Table IV. The per cent P in the first cut of Trial 1 shows a remarkable 6-fold variation and a 2 or 3-fold variation for the other cuts. Although these data are the mean values of the P_0, P_1 and P_2 treatments, application of P had a negligible effect on the per cent P in the clovers, even when it was as low as 0.10 per cent. The application of lime or molybdenum generally produced a reduction in per cent P

which is more marked in Trial 1 than in Trial 2. The increased yields of dry matter consequent on the larger amounts of N fixed by application of lime or molybdenum were accompanied by corresponding decreases in per cent P, and in Trial 1 particularly, the yields of P were very similar for all treatments. This relative independence of P-uptake on the yield of dry matter is clearly shown in Fig. 3. It has been shown by Walker, Edwards *et al.* [2,3]) that the application of fertiliser N to pastures on soils deficient in P reduces the per cent P of the herbage, whereas on soils rich in P, the per cent P increases. Both the soils in these trials were fairly deficient in "available" P, and if the increased amount of N fixed by the clovers on application of lime or molybdenum can be compared with the effect of fertiliser N on non-legumes, a fall in the per cent P is to be expected. Apart from the L_0M_0 treatments in some cases, the yields of P were similar whether lime were applied or not; and where molybdenum was applied in the absence of lime, yields of P were similar to those on the limed soils. From a comparison of the P_1 and P_2 yields with the P_0 yields, the amount of P recovered in the two cuts in the tops was only a fraction of the P applied (10–12 per cent in all cases). All the evidence points to P being almost equally "available" under all treatments. There is certainly little to indicate that lime has made P more "available" or reduced "fixation".

(*d*) Molybdenum. The molybdenum data are presented in Table V. In the first cut of Trial 1, all the no-lime and no-molybdenum

TABLE V

Yields of Mo (μg per pot) and ppm Mo in dry matter

Trial 1

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	.1(.10)	.4(.12)	.6(.08)	.8(.35)	2.0(.27)	3.5(.43)
M_1	.2(.12)	1.4(.28)	2.4(.33)	2.5(.44)	3.2(.44)	4.7(.67)
M_2	.1(.05)	1.0(.16)	2.3(.35)	8.5(1.29)	3.0(.39)	7.4(1.01)

Trial 2

	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	.7(.65)	1.1(.60)	1.4(.50)	2.3(1.07)	2.6(.42)	2.6(.38)
M_1	1.1(1.16)	1.0(.40)	2.3(.60)	2.0(.45)	2.9(.33)	3.6(.47)
M_2	.6(.46)	1.1(.37)	2.6(.47)	3.2(.64)	3.4(.43)	3.8(.49)

treatments contained about 0.1 ppm Mo in the dry matter, and this is often considered to be about the deficiency level although there is considerable discrepancy in the literature (Anderson and Oertel[7]), and Rossiter[22])). Where lime and molybdenum were applied together in this cut the Mo-content was increased. In Cut 2, the Mo-contents were higher for all treatments, and molybdenum applications were particularly effective in increasing the concentration. The yields of molybdenum were increased markedly by lime or molybdenum treatments.

In Trial 2, the Mo-contents were usually considerably higher than in Trial 1, and whilst the trends are not always the same, lime and molybdenum treatments tended to reduce the Mo-content. The most likely explanation of this, as will be seen when manganese is considered, is that both lime and molybdenum treatments effect reductions in the Mn-content of the clovers below the possible toxic level, and manganese toxicity or Mn–Mo antagonism rather than molybdenum deficiency was the real cause of poor growth in the Trial 2 soil. The yields of molybdenum are plotted against yields of dry matter in Fig. 3, and in Fig. 4 the ppm Mo in the dry matter have been plotted against yields of N. In both cases

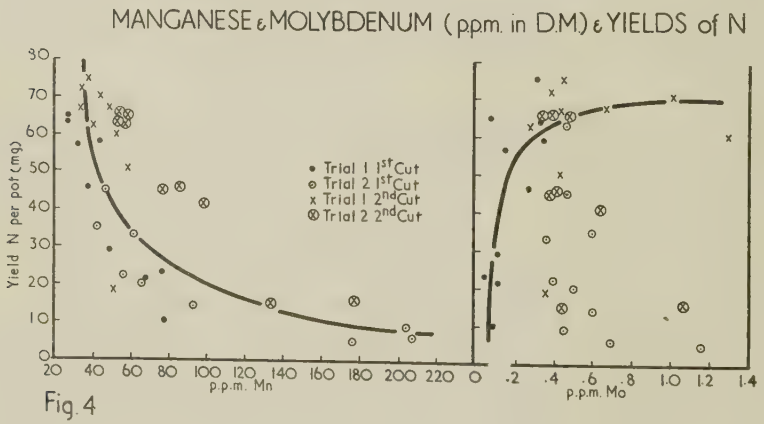

Fig. 4

the curve has been put through the results for Trial 1, and it can be seen that most of the Trial 2 plots lie well below the curves, indicating that less N has usually been fixed in Trial 2 than in Trial 1, for a given Mo-content. By contrasting this with the plot of ppm Mn against yields of N in Fig. 4, it would appear that high

Mn-contents have not affected uptake of Mo, but have reduced its effectiveness in the process of N-fixation. In Trial 1, where toxic manganese does not appear to have been an important factor, about 0.1 ppm Mo in the dry matter seems to be the critical concentration.

TABLE VI

Yields of Mn (μg per pot) and ppm Mn in dry matter

	Trial 1					
	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	26(77)	52(48)	70(27)	36(50)	97(40)	83(33)
M_1	42(67)	64(38)	67(27)	110(58)	93(38)	119(48)
M_2	53(76)	66(32)	97(44)	115(52)	84(33)	107(44)
	Trial 2					
	Cut 1			Cut 2		
	L_0	L_1	L_2	L_0	L_1	L_2
M_0	71(207)	59(93)	59(66)	128(177)	175(85)	178(77)
M_1	56(176)	46(56)	55(42)	125(133)	171(58)	131(52)
M_2	87(204)	63(61)	85(46)	162(98)	153(57)	152(58)

(*e*) Manganese. Yields and content of Mn are shown in Table VI. There is a considerably higher level of Mn in Trial 2 for both cuts. In both trials the application of lime has caused a reduction in the ppm Mn. Molybdenum treatments have generally given lower Mn-contents in Trial 2, but this effect is not so obvious in Trial 1. The yields of Mn are fairly uniform within each cut of Trial 2 considering the large variation in ppm Mn. In Trial 1 in spite of a lowered Mn content, lime has given higher yields of Mn than no lime, but apart from the L_0M_0 treatments the variation in the yields of Mn are not pronounced.

Kipps [15]) has noted that the tolerance of lucerne to manganese toxicity is probably in the order of 150 200 ppm in the dry matter, and in Trial 2, the Mn-content is within this range for most of the L_0 treatments in Cut 1. For the second cut in Trial 2, the L_0 treatments all contain more than about 100 ppm Mn, and the L_1 and L_2 treatments in the absence of molybdenum contained about 80 ppm Mn. Considerably less N was fixed with these treatments and the clovers in these pots were all yellowish green. As shown in Fig. 4 the yields of N show a marked negative correlation with the ppm Mn, and it would appear that N-fixation is affected by Mn-toxicity only above about 60–80 ppm Mn.

DISCUSSION

The acidity complex

Hewitt [14]) has recently discussed the problem of soil acidity and it is becoming more and more obvious that actual calcium deficiency is rare except on very acid soils. Plant [21]) has recently demonstrated this in the field with various nonlegumes. The commonest primary causes of poor crop growth on acid soils would appear to be toxicities of manganese and aluminium or molybdenum deficiency. There is as far as we know very little published work on aluminium toxicity in clovers grown in soils, and it was not possible to analyse the clovers in these trials for aluminium because of the low yields from the unlimed soils. Molybdenum appears to be rendered more available by liming many acid soils, and as lime is normally considered to convert Mn^{++} to higher oxides thus making it less soluble, applications of lime will usually have the dual effect of correcting both Mn-toxicity and Mo-deficiency (Oertel, Prescott and Stephens [20]). Lime is credited with so many other beneficial effects (biological, physical and reduction of P-fixation), it would generally be agreed that applications of lime are the most effective treatments for acid soils. Indeed this view is so widely accepted that in humid-temperate climates it is the normal aim in advisory work when estimating the "lime requirement" of soils to assess the amount of lime needed to raise the pH to 6.0–6.5 (Walker [27]). This policy has recently been questioned by Åslander [9] [10]), who has succeeded in growing good crops on moderately acid soils by the heavy use of dung and fertilisers. It is possible in these cases that the molybdenum contained in the dung corrected molybdenum deficiency, which may have been primarily responsible for the poor crops, and the nitrogen and other fertilisers applied would then be expected to give enhanced yields.

In Trial 1, an application of sodium molybdate at 2 ounces per acre eventually gave yields of white clover as high as 1½–3 tons of $CaCO_3$ per acre, and from the analytical data presented it would appear that molybdenum deficiency brought about primarily by the low soil pH was responsible for the poor growth of clovers, as applications of lime or molybdenum both increased the molybdenum uptake and the amount of N fixed. In Trial 2 manganese toxicity was the most like lycause of poor growth as the Mo-contents were relatively high in all treatments. Molybdenum still effected a

marked improvement in the absence of lime, but both lime and molybdenum were needed to give maximum yields and reduce the manganese content to below 60 ppm. It is possible that heavier dressings of Mo would produce the same result. As has been shown in the case of nonlegumes (Millikan [18]) there appears to be a definite Mn -Mo antagonism in the case of legumes *). Anderson and Arnot [4]) on a soil where white clover responded more to lime than molybdenum, found that manganese sulphate caused a marked reduction in yield, while sodium molybdate counteracted this effect (the only symptoms were of N-deficiency). Earlier, Anderson [3]) had suggested that Millikan's views on the similarity between molybdenum deficiency and manganese toxicity were probably incorrect. It would appear however where manganese contents are high, that there is some interference with the role of molybdenum in N-fixation, which can be overcome to some extent by further applications of molybdenum; N-fixation and dry matter production are thus increased and the manganese content in the dry matter falls.

Trial 2 soil contained more exchangeable and easily reducible manganese and more "available" molybdenum than Trial 1, and this agrees very well with the plant analytical data. Davies [12]) has suggested that the pH of a soil as well as its "available" molybdenum content [13]) must be considered when assessing possible responses to molybdenum, and at low pH levels a higher "available" molybdenum is more necessary than at higher pH levels. This could be because of the higher manganese availability at lower pH levels. Davies [12]) also describes a liming trial in which yields of sub clover were inversely related to manganese contents. Whilst molybdenum may increase yields of clover on acid soils high in exchangeable and easily reducible Mn, it would appear preferable to immobilise some of this Mn by liming, because higher applications

*) As this paper goes to press, we have just received a paper by Mulder and Gerretsen [19]) where it is suggested that Mn–Mo antagonism in the case of clovers is unlikely. The authors however overlook the fact that straight Mo-deficiency may arise without complications from Mn-toxicity. In other cases responses may be obtained from Mo where the real problem is one of Mn–Mo antagonism. The problem is similar but not identical to Mg-deficiency, which may be caused by an absolute deficiency of Mg or say a K–Mg antagonism. In this case however K reduces Mg-uptake, but with Mn–Mo antagonism it would appear that Mo is taken up, but immobilised within the plant.

of molybdenum may be needed than in cases of straight molybdenum deficiency, with consequent risks to stock health, particularly if the soil is limed subsequently.

In neither case over the period of these two trials was calcium apparently of importance *per se*, although as suggested by Australian work, lime may enhance rapid nodulation of clovers on acid soils and thus assist in early establishment and growth. Although molybdenum in the absence of lime lowered the per cent Ca in the clovers, it would in practice by increasing clover production in a grass-clover sward, increase the overall per cent Ca in the mixed herbage, as clovers contain considerably more Ca than grasses.

Whilst the empirical approach to the problem of liming has been very effective, it appears that appropriate soil and plant analyses would permit a more fundamental approach, and this is of particular value where liming may be difficult or impossible on account of topography or cost.

Phosphate "fixation" on acid soils

Recent Australian work suggests that the significance of P-fixation on acid soils has been over-estimated. It is obvious that where lime corrects molybdenum deficiency or reduces the effect of a Mn–Mo antagonism, responses may be obtained to phosphate which would not be obtained in the absence of lime. If molybdenum deficiency is corrected by applications of molybdenum however, normal phosphate responses may be obtained, suggesting that phosphate is equally available at the lower pH. This is further supported by the analytical data presented in this paper where in the most striking example (Cut 1, Trial 1), yields of dry matter at L_2M_2 were six times greater than those at L_0M_0. The P-contents however were 0.12 and 0.68 per cent respectively and the yields of P were similar. As far as the plant was concerned P was just as available in the absence of lime as in its presence. Whilst long-term residual-P experiments are urgently needed to clarify our knowledge of P-fixation and availability, it is clear that laboratory experiments on P-fixation may be quite misleading in some cases. In this context we have recently had the following very striking example, which will be considered in greater detail elsewhere. In trials on the establishment and growth of clovers with all combinations of gypsum (S), monocalcium phosphate (P) and sodium

molybdate (Mo), S gave a marked depression in clover yields on one soil. The soil was very deficient in available P and clovers responded well to P alone. S + P gave much lower yields similar to the control, whilst S + P + Mo restored the yield to the level obtained from P alone. The control clovers contained 205 ppm Mn in the dry matter, and application of S increased this to 275 ppm Mn; the application of Mo presumably corrected the Mn–Mo antagonism which had been intensified by the application of S. This effect of gypsum on the Mn-uptake of lucerne has also been noted by Kipps [15]), and by Schmehl *et al.* [23]). The application of superphosphate (S + P) on this very P-deficient soil would have been ineffective, and may have lead to the assumption that P was rendered highly unavailable by P-fixation. If the soil had been limed, thereby reducing the availability of Mn, superphosphate responses may have been normal, thus supporting the concept that lime makes P more available, whereas its main effect in this case would have been the correction of Mn-toxicity or the reduction of the effect of a Mn–Mo antagonism. In all the other trials, P alone gave very much lower yields than S + P because of S-deficiency. It is obvious that care is needed in the design and interpretation of P trials, and that plant and soil analyses may be very revealing.

The analytical data presented on P-uptake have some interesting implications. Phosphate would appear to have behaved in general like any other water-soluble anion (*e.g.* NO_3^-) present only in limited amount, as over the same period of time plants varying greatly in dry matter production absorbed similar quantities of P, with what amounts to luxury consumption on the one hand and deficiency levels on the other. As fast as P was removed from solution by the clovers more came into solution, and the rates and amounts involved would appear to have been similar under the various conditions.

SUMMARY

1. Molybdenum, phosphate and lime interactions on the yield and composition of white clover were followed on two related soils of pH 5.3. The soil formed under higher rainfall conditions contained rather more available molybdenum and much more exchangeable and easily reducible manganese; exchangeable calcium was very low in both soils.

2. On the lower rainfall soil, molybdenum deficiency was the primary

cause of poor clover growth, as molybdenum applications gave yields as high as half or full lime requirement rates. Manganese contents of the dry matter were always less than 80 ppm. In the other soil, manganese toxicity was probably the main cause of poor growth as the molybdenum and manganese contents were considerably higher. There was evidence of a Mn–Mo antagonism affecting the role of Mo in N-fixation. No Ca-deficiency was evident, the only symptoms recorded being those of N-deficiency.

3. There was a high positive correlation between yields of dry matter, N and Ca, whereas uptake of P was almost independent of dry matter yields.

4. The classical concept of the "lime-requirement" of soils and P-fixation on acid soils have been discussed in the light of these and other results. It is suggested that a more fundamental approach to problems of acidity is now possible; accepted views of P-fixation and availability on some acid soils are challenged and it is suggested that long-term residual-P trials are urgently needed to qualify statements based largely on laboratory experiments. Particular attention would have to be paid to the forms of phosphate used, and the correction of other mineral deficiencies or excesses.

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LITERATURE CITED

1) Adams, A. F. R., Molybdenum and Legumes. Proc. 14th Conf. New Zealand Grassland Assoc. 202–210 (1952).
2) Anderson, A. J., Molybdenum in relation to pasture improvement in S. Australia. J. Counc. Sci. Ind. Research Australia **19**, 1–15 (1946).
3) Anderson, A. J., Molybdenum and other fertilisers in pasture development on the S. Tablelands of N. S. Wales. J. Australian Inst. Agr. Sci. **14**, 28–33 (1948).
4) Anderson, A. J. and Arnot, R. H., Fertiliser studies on Basaltic Red Loam Soil from the Lismore district, N.S. Wales. Australian J. Agr. Research **4**, 29–43, (1953).
5) Anderson, A. J. and McLachlan, K. D., The residual effect of P on soil fertility and pasture development on acid soils. Australian J. Agr. Research **2**, 377–400 (1951).
6) Anderson, A. J. and Moye, D. V., Lime and molybdenum in clover development on acid soils. Australian J. Agr. Research **3**, 95–110 (1952).
7) Anderson, A. J., and Thomas, M. P., Plant responses to molybdenum as a fertiliser. I. Molybdenum and symbiotic N-fixation. Counc. Sci. Research Australia Bull. no 198.

8) Anderson, A. J., and Oertel, A. G., II Factors affecting the response of plants to molybdenum. Ibid.
9) Åslander, A., Soil fertilization and liming. Soil Sci. **74**, 181–195 (1952).
10) Åslander, A., Standard fertilization and the quality of crops. Soil Sci. **74**, 431–442 (1952).
11) Davies, E. B., Holmes, G. A. and Lynch, P. B., Pasture responses to molybdenum top-dressing in Otago and Southland. New Zealand J. Agr. **83**, 247 (1951).
12) Davies, E. B., Minor element problems in New Zealand. Trans. Int. Congr. Soil Sci. Dublin, Vol. **1**, 167–179 (1952).
13) Grigg, J. L., A rapid method for the determination of Mo in soils. The Analyst **78**, 470–473 (1953).
14) Hewitt, E. J., A biological approach to the problems of soil acidity. Trans. Intern. Congr. Soil Sci. Dublin, Vol. **I**, (1952).
15) Kipps, E. H., The calcium manganese ratio in relation to the growth of lucerne at Canberra. A.C.T. J. Counc. Ind. Sci. Research Australia **20**, 176–189 (1947).
16) Leeper, G. W., The forms and reactions of manganese in the soil. Soil Sci. **63**, 79–94 (1947).
17) Lobb, W. P., Molybdenum investigations in N. Otago. New Zealand J. Agr. **84**, 356–57, (1952).
18) Millikan, C. R., Effects of molybdenum on the severity of toxicity symptoms in flax induced by an excess of either manganese, zinc, copper, nickel or cobalt in the nutrient solution. J. Australian Inst. Agr. Sci. **13**, 180–186 (1947).
19) Mulder, E. G. and Gerretsen, F. C., Soil manganese in relation to plant growth. Advances in Agron. **4**, 222–277 (1952).
20) Oertel, A. C., Prescott, J. A. and Stephens, C. G., The influence of soil reaction on the availability of molybdenum to sub. clover. Australian Sci. **9**, 27–28 (1946).
21) Plant, W., An analysis of the acid soil complex by the use of indicator plants. Plant and Soil **5**, 54–66 (1953).
22) Rossiter, R. C., Growth of subterranean clover as affected by lime, molybdenum and potassium. Australian J. Agr. Research **3**, 244–258 (1952).
23) Schmehl, W. R., Peech, M. and Bradfield, R., Causes of poor growth of plants on acid soils and beneficial effects of liming: 1. Evaluation of factors responsible for acid-soil injury. Soil Sci. **70**, 393–410 (1950).
24) Spencer, D., The effect of calcium and soil pH on nodulation of *T. subterraneum* L. clover on a yellow podsol. Australian J. Agr. Research **1**, 374–381 (1950).
25) Stout, P. R., Meagher, W. R., Pearson, G. A. and Johnson, C. M., Molybdenum nutrition of crop plants. I. Plant and Soil **3**, 51–87 (1951).
26) Truog, E., The determination of readily available P of soils. J. Am. Soc. Agron. **22**, 874–882 (1930).
27) Walker, T. W., The estimation of the lime requirements of soils. J. Soil Sci. **3** 261–276 (1952).
28) Walker, T. W., Molybdenum responses on New Zealand pastures. World Crops **5**, 267–269 (1953).
29) Walker, T. W., Edwards, G. H. A., Cavell, A. J. and Rose, T. H., The use of fertilisers on herbage cut for conservation. 2. J. Brit. Grassland Soc. **7**, 135–150 (1952).

APPENDIX

TABLE VII

Yields of dry matter in grams per pot (mean values)

Trial 1 Cut 1

	M_0	M_1	M_2	L
L_0	0.34	0.64	0.69	0.56
L_1	1.10	1.68	2.06	1.61
L_2	2.57	2.45	2.21	2.41
S.E.	± 0.15			±0.09

	L_0	L_1	L_2	P
P_0	0.44	1.68	2.30	1.47
P_1	0.61	1.56	2.54	1.57
P_2	0.62	1.59	2.40	1.53
	± 0.15			±0.09

	P_0	P_1	P_2	M
M_0	1.28	1.36	1.36	1.33
M_1	1.68	1.47	1.29	1.48
M_2	1.46	1.89	1.62	1.66
	± 0.15			±0.09

Trial 1 Cut 2

	M_0	M_1	M_2	L
L_0	0.72	1.89	2.22	1.61
L_1	2.41	2.41	2.52	2.45
L_2	2.69	2.48	2.46	2.54
S.E.	± 0.27			±0.11

	L_0	L_1	L_2	P
P_0	1.26	2.21	2.22	1.90
P_1	1.65	2.56	2.76	2.32
P_2	1.93	2.57	2.49	2.33
	± 0.27			±0.11

	P_0	P_1	P_2	M
M_0	1.68	2.13	1.86	1.89
M_1	1.95	2.33	2.50	2.26
M_2	2.07	2.51	2.63	2.40
	± 0.27			±0.11

Trial 2 Cut 1

	M_0	M_1	M_2	L
L_0	0.34	0.31	0.44	0.36
L_1	0.63	0.82	1.05	0.83
L_2	0.91	1.30	1.84	1.35
S.E.	± 0.05			±0.02

	L_0	L_1	L_2	P
P_0	0.30	0.82	1.19	0.77
P_1	0.39	0.79	1.27	0.82
P_2	0.41	0.88	1.60	0.96
	± 0.05			±0.02

	P_0	P_1	P_2	M
M_0	0.59	0.66	0.63	0.63
M_1	0.70	0.86	0.87	0.81
M_2	1.01	0.93	1.39	1.11
	± 0.05			±0.02

Trial 2 Cut 2

	M_0	M_1	M_2	L
L_0	0.72	0.94	1.66	1.11
L_1	2.06	2.94	2.68	2.56
L_2	2.30	2.52	2.62	2.48
S.E.	± 0.18			±0.08

	L_0	L_1	L_2	P
P_0	0.59	2.06	2.37	1.67
P_1	1.08	2.69	2.36	2.04
P_2	1.64	2.92	2.70	2.42
	± 0.18			±0.08

	P_0	P_1	P_2	M
M_0	1.19	1.78	2.11	1.73
M_1	1.88	2.15	2.38	2.14
M_2	1.96	2.22	2.78	2.32
	± 0.18			±0.08